INDIANA STATE DEPARTMENT OF HEALTH

INDIANA CERTIFICATION STANDARDS FOR DRINKING WATER MICROBIOLOGY LABORATORIES

July 2005

Adapted from the Fifth Edition of the
US EPA Manual for the Certification of
Laboratories Analyzing Drinking Water

ISDH
LABORATORY RESOURCE CENTER
ENVIRONMENTAL LABORATORY DIVISION
LABORATORY IMPROVEMENT SECTION
INDIANAPOLIS, INDIANA 46202

Disclaimer

This manual has been reviewed and approved for publication by the Indiana State Department of Health Laboratory Resource Center. the Laboratory Certification Office of the Environmental Laboratory Division and the Laboratory Improvement Section.

The mention of commercial products does not constitute endorsement by the ISDH.

Regulatory changes made subsequent to the publication of this manual take precedence over the contents.

Note: References to *Standard Methods for the Examination of Water and Wastewater* are to the 20th edition (1998)

Table of Contents

Chapter I

Introduction

In 1974, Congress enacted the Safe Drinking water Act to protect the public from the consumption of contaminated drinking water. It directed the US Environmental Protection Agency (EPA) to establish minimum drinking water standards for compliance by public water systems serving at least 25 persons or having at least 15 service connections. It was further amended in 1986 and authorized the EPA to publish several regulations (Code of Federal Regulations) in order to implement the amendments. Mandatory guidelines were set to regulate key contaminants, to require the monitoring of unregulated contaminants, to establish standards for water treatment technologies, to enforce compliance and to promote protection of ground water sources.

The National Primary Drinking Water Regulations (NPDWR) (40 CFR 141and 142) defined the criteria and procedures to insure compliance and require that States have the responsibility to avail certified public or private laboratories to perform compliance sample testing. It requires States to establish and maintain a program for the certification of laboratories performing analysis of drinking water samples for the presence of contaminants. It involves auditing the laboratories and reviewing Performance Evaluation data. Consequently, the Indiana Primary Drinking Water Regulations (IPDWR) were passed to reflect and meet the requirements of the national regulation.

Chapter II describes Certification Guidelines and Chapter III describes Critical Elements for Microbiology. Appendices include frequently used abbreviations and definitions, guideline for a Quality Assurance (QA) Plan, approved analytical methods for Microbiology, TCR/SWTR and important contact numbers/ websites. Also attached is the audit checklist used for on-site evaluations.

1. Authority

1.1 To comply with 40 CFR 142.10, a state must establish and maintain a program for the certification of laboratories conducting analytical measurements of drinking water contaminants pursuant to the requirements of the state primary drinking water regulations including the designation by the state of a laboratory certification officer certified by the USEPA unless all analytical measurements are conducted at State laboratories and certified by EPA. (40 CFR 142.10(b)(3)(i)).

1.2 Rule 327 Indiana Administrative Code (IAC) 8-2-8.7 of IPDWR includes the following provision: The measurements for Total coliform, Fecal coliform (Escherichia coli), and Heterotrophic Plate Count must be conducted by a laboratory certified by the Indiana Department of Environmental Management (IDEM) commissioner/ designee or by USEPA.

1.3 Under an agreement with the IDEM, the Indiana State Department of Health (ISDH) conducts surveys and determines certification status of laboratories that perform the microbiological examination of public drinking water.

2. Purpose

2.1 This manual is intended to help implement 327 IAC by defining and specifying criteria and procedures for certifying environmental microbiology laboratories, and to:

2.2 Establish the guidelines and requirements for laboratory personnel, facilities, equipment and supplies, general laboratory practices, analytical methodology, sample collection, handling and preservation, quality assurance, records and data reporting and action-response to laboratory results;

2.3 Establish the enforcement actions that IDEM and ISDH must follow to ensure that all certified laboratories are in compliance with the guidelines;

2.4 Establish the understanding that all laboratories enrolled with this program have entered into an agreement and must submit to and comply with all the requirements defined in this document, with subsequent penalty(ies) for violations.

Chapter II

Certification Guidelines

Any laboratory that analyzes drinking water compliance samples is considered a drinking water laboratory for the purpose of certification. All such laboratories must be certified by the State or EPA. To be eligible to analyze compliance samples under the Safe Drinking Water Act, water laboratories should meet the minimum criteria specified in this manual.

1. Certification Process

1.1 Laboratories seeking certification should forward all inquiries to the ISDH Laboratory Certification Office (See Appendix E). The process begins when the laboratory director makes a formal application to the Microbiology laboratory certification officer (CO). The application may be a request for first-time certification, a request for certification to analyze additional or newly regulated contaminants, or a request to reapply for certification after correction of deficiencies, which resulted in the downgrade/revocation of certified status.

1.2 Response to the formal application should be given within 30 days. The laboratory should verify that they plan to analyze drinking water samples when they request certification. If the laboratory does not plan to, ISDH may choose not to conduct the survey or issue certification. Laboratories seeking first time certification are sent an information package containing registration and pre-survey forms, a copy of this certification manual, a copy of the audit checklist used for the onsite evaluation, the schedule of QC/maintenance requirements for equipment and supplies, a list of accredited microbiology performance evaluation providers and other forms, record logs or items to help the laboratory get started.

1.3 The laboratory director must provide documentation to show the laboratory's ability to meet all certification requirements. The laboratory must submit the following for review:

- The registration and pre-survey forms
- The laboratory Quality Assurance Plan (See Appendix B)
- Standard Operating Procedures for the methods to be used in analyzing compliance samples, including the SOP for the laboratory's own calibration and maintenance of equipment
- At least three months of QC records
- Two most recent Performance Evaluation (PE) results
- Description of personnel qualifications (education, training, technical knowledge and experience for the assigned functions)

1.4 When it has been determined that the laboratory is ready, an on-site survey by ISDH certification officers will be scheduled. The CO will contact the laboratory to arrange a mutually acceptable date for the survey.

1.5 The protocol for the onsite survey shall be conducted in accordance with EPA guidelines. The CO must carry and show an official identification. After preliminary introduction to Senior staff and other personnel involved in the laboratory operation, the CO may tour the facility first and provide an overview of the survey process, discuss safety issues and other concerns. The CO will then proceed to review records/ documentation and interview or observe analyst/analysts regarding the items being considered in the evaluation. The analyst maybe asked to explain a procedure or test. The CO may inquire about equipment maintenance, QC's, sample processing, data handling, reports,etc. If deviations are noted, it will be pointed out during the audit, corrective action should be specified at that time citing the reason and state requirement or regulation to be followed.

1.6 After the survey, the CO should conduct an exit briefing, attended by key personnel, in which the findings will be reviewed, deviations discussed and the corrective actions cited. Status recommendation and appeal procedure, if applicable, may also be explained at this time.

1.7 After the onsite survey and a review of PE results, the CO will classify the status of the laboratory according to the following types:

Certified - a laboratory that meets the minimum requirements of the guidelines in this manual and all applicable regulatory requirements such as the NPDWR and IPDWR. This certification shall be valid for three years, subject to revocation and other requirements.

Provisionally Certified - a laboratory which has deficiencies but can demonstrate its ability to produce valid data within the acceptance limits determined by the ISDH Certification Office. The laboratory may be given up to 6 months to correct its deviation(s) afterwhich the provisional status is removed. If the deficiency is not corrected within the timeline, certification shall be revoked.

Not Certified - a laboratory possessing major deficiencies which would not allow the laboratory to consistently produce valid data within specified acceptance limits. The laboratory may be given up to six months to correct the deviations afterwhich it may apply for a re-certification.

Interim Certification – this is granted in certain circumstances when it is impossible or unnecessary to perform an on-site audit. Interim certification status may be granted only when the CO is satisfied that the laboratory has met the requirements (appropriate instrumentation, approved methods, adequately trained analyst) and has satisfactorily analyzed PE samples for the analyte in question. An example of this is when the laboratory is changing to a new EPA approved method in place of a previously certified method which uses the same basic principle. The CO will review the parallel study records, QC control data, SOP for the new procedure and the PE results. The CO will conduct an on-site audit as soon as possible but in no case later than three years. This status is also granted in situations when a laboratory requests certification for the analysis of additional contaminants using a method for which it has already been certified.

1.8 The CO drafts an evaluation report stating any deviation, any recommendation and the certification status recommendation and forwards it to the Laboratory Resource Center (LRC) for review. If the LRC concurs with the recommendations, the CO will send an official report to the laboratory. A copy of the full audit may also be attached to the report. A timeline will be specified for the correction of deviations, if any. A description of corrective action plan for the noted recommendations should also be submitted for the CO to review.

1.9 When all the requirements are met, the ISDH Director of Laboratory Services will review the report and proper documentation and determines the certification status of the laboratory. If the evaluation was satisfactory, a Letter of Certification or a Certificate of Approval, which can be displayed, will be signed by the ISDH LRC Director and issued to the laboratory.

1.10 In order to assess validity of certification between on-site surveys, laboratories are required to satisfactorily pass proficiency testing for each of the methods used in testing compliance samples and register annually so that key personnel, facility or method changes may be monitored. Laboratories may also be required to submit copies of any records or documentation deemed necessary by the certification office (e.g. reagent water suitability testing results).

2. Requirements for Maintaining Certification

2.1 On-site evaluation will be conducted at least once every three years. No unannounced on-site survey shall be performed unless unusual circumstances warrant it. If the laboratory undergoes a major change, or consistently fails PE samples, the CO might consider conducting an evaluation before the usual three-year period has elapsed.

2.2 The laboratory evaluation will be based on the competence of staff; working conditions (including the adequacy of space; lighting, equipment, and supplies); analytical methods used; quality control procedures; sampling procedures and maintenance of all required records; action response to laboratory results; and compliance with the requirements of these guidelines.

2.3 The laboratory must perform only the methodology specified in the Total Coliform Rule, Surface Water Treatment Rule and/or Ground Water Rule. Approved methods are listed in Appendix C.

2.4 The laboratory must be certified for all the analytical methods it uses for compliance purposes and be certified for at least one Total coliform method, one Fecal coliform or *E. coli* method and the Heterotrophic Plate Count method for enumerating heterotrophic bacteria. The laboratory must also have a backup procedure or be certified for a second method if certain water samples cannot be analyzed with the methods normally used.

2.5 The laboratory must satisfactorily analyze annual PE samples for each category of certified method used in testing compliance samples. The PE provider should send the report directly to the ISDH CO for evaluation. Furthermore, the laboratory should be able to document that the analyst performing the PE assay is a laboratory personnel who routinely analyzes compliance samples.

2.6 Laboratories must notify ISDH Certification Office in writing, within 30 days, of major changes in personnel, equipment, or location. A major change that results in the loss of capacity to produce valid data will result in the downgrade or revocation of certification.

2.7 Laboratories are required to submit the annual registration form at the end of the year to update and provide accurate listing to the public and other agencies needing the information.

3. Downgrading or Revoking Certification Status

3.1 **A laboratory may be downgraded to Provisional Status for any of the following reasons:**

- Failure to maintain the required standard of quality based on the onsite evaluation or these guidelines.
- Failure to analyze PE samples annually within acceptance limits.
- Failure to submit the annual completed registration form.
- Failure to report compliance data to the client or public water system in a timely manner thereby preventing them from complying with Federal and/or State regulations and endangering public health. Data that may cause the system to exceed the Maximum Contaminant Level (MCL) should be reported as soon as possible.
- Failure to notify ISDH LRC within 30 days of a major change which could impair analytical capability (in personnel, equipment or laboratory location).

3.2 **Procedure for Downgrading to Provisional Status**

- If a laboratory is being considered for a provisional status downgrade, the ISDH LRC must notify the laboratory director, in writing, using certified mail. The laboratory will be notified of its right to appeal the decision at this time. The ISDH will request that the laboratory notify its clients of its status in writing, and submit verification that this has been done. IDEM will also be notified in writing of this change.
- Within 30 days of receipt of the letter, the laboratory director must notify the CO, in writing, of its intent to regain full approval by specifying the corrective action to be taken.
- A laboratory may be given up to 3 months to correct procedural, administrative or personnel deficiency with a possible 3 month extension (for a major equipment replacement, for example).
- A laboratory that failed a PE sample within the acceptance limits must successfully analyze a second PE sample before the provisional status is removed. The laboratory may continue to analyze compliance samples but should notify its clients of its downgraded status and provide that information, in writing, on any report.

3.3 **A laboratory may have certification revoked for the following reasons:**

- Failure to satisfy ISDH LRC that the laboratory has corrected the deviations identified during the on-site evaluation within the given time.
- Submission of a PE sample to another laboratory for analysis and reporting the data as its own
- Falsification of data or other deceptive practices
- Failure to use analytical methodology specified in the regulations
- Consistently failing to satisfactorily analyze PE samples within the acceptance limits
- Persistent failure to report compliance data to the public water system or IDEM drinking water program in a timely manner thereby preventing compliance with Federal and/or State regulations and endangering public health. Data which may cause the system to exceed an MCL should be reported as soon as possible.

3.4 **Procedure for Revocation**

- If the CO finds that an emergency situation warrants immediate action, summary suspension may be ordered pending revocation proceedings. An emergency situation warrants immediate action if there is substantial risk to public health, safety, or welfare resulting from laboratory deficiencies that compromise the analytical results obtained.
- In non-emergency situations, the ISDH LRC will notify the laboratory and IDEM in writing, by certified mail, of the intent to revoke certification. The ISDH will request the laboratory stop testing drinking water. The ISDH will request that the laboratory notify its clients of its status in writing, and submit verification that this has been done. IDEM will be notified immediately of the laboratory's status.
- The laboratory may challenge this decision in writing to the Indiana State Department of Health, Secretary, 2 North Meridian, Indianapolis, IN 46204 within 15 days of receipt of revocation notice. If the laboratory does not wish to challenge the decision, it must inform the CO of its intent to accept the revocation of certification within 15 days of receipt of letter of revocation. The laboratory director shall return the laboratory certificate with the letter of revocation acceptance. If the laboratory does not reply, the CO will request clarification of the laboratory's intentions.
- The notice of appeal must be supported with an explanation of the reasons for the challenge and must be signed by the highest authority of the laboratory such as the president/owner for a commercial laboratory, or the laboratory director/supervisor in the case of a public laboratory.
    - The petitioner must be a person to whom the determination is specifically directed.
    - The petitioner is aggrieved or adversely affected by the determination.
    - The petitioner is entitled to review under any law.
- The State Hearing Officer will make a decision regarding the appeal and notify the laboratory in writing with the results of the appeal. Denial of appeal will result in immediate revocation of the laboratory's certification. Once certification is revoked, a laboratory may not analyze compliance samples until certification has been reinstated.
- If the appeal is determined to be valid, ISDH LRC should take the appropriate measures to conduct a reevaluation and notify the laboratory in writing, by certified mail, within 30 days.

3.5 Withdrawal or Cancellation of Certification

Any certified laboratory may cancel or withdraw its certification by notifying the Certification Officer in writing. The letter must be signed by the laboratory director and the Letter of Certification and the Certificate of Approval enclosed. The laboratory will be removed from the list of certified laboratories published in the state and EPA websites. ISDH LRC will issue a letter to the laboratory confirming the withdrawal and send a copy of the letter to IDEM.

3.6 Reinstatement of Certification

- Certification may be reinstated when and if the laboratory can demonstrate to the certifying authority that the deficiencies causing downgrading or revocation of certification have been corrected.

- The certification officer may request another on-site evaluation, analysis of additional PE samples, or other measures deemed appropriate by the certification office.

4. Reciprocity

4.1 ISDH may elect to enter into a reciprocal agreement with other states in recognition of mutually acceptable certification programs. Out-of-state laboratories will have to meet the same requirements as Indiana Certified Laboratories. The following items will be requested for review by the State CO:

- Copy of laboratory's current home state certification
- Copy of laboratory's recent home state on-site evaluation
- Copy of corrective actions to any cited deviation and the home state certifying authority's response
- Copy of the last two PE studies
- Copy of the laboratory's SOP and QA Plan
- Copy of laboratory personnel educational and training records
- Completed Indiana Drinking Water Laboratory Registration form

4.2 At the time of this Certification Manual's final approval, there is no provision for the use of a third party auditing agent

5. Other Considerations for Certification

5.1 The laboratory must have adequate supervision and staffing with the necessary education or training for their assigned positions.

5.2 The laboratory director/manager should be a qualified professional with the technical education and experience to manage the size and type of laboratory. The laboratory director is responsible for ensuring that the requirements for the personnel are met and that all data reported meet the QA criteria and regulatory requirements.

5.3 The QA Officer/manager should have a bachelor's degree in science, with proper training and adequate experience in QA/QC principles and a working knowledge of the statistics of the laboratory QC and a basic understanding of the analytical methods used. The QA Officer should be independent from the laboratory management if possible and be responsible for keeping the QA plan up to date.

5.4 The laboratory must have a written Quality Assurance plan in place and updated as necessary. All laboratory personnel must be familiar with the contents of the plan. The Laboratory QA plan must be available for review prior to or during the on-site visit. See Appendix B for the essential elements of a QA Plan.

5.5 The laboratory must have a chain-of-custody procedure for use in case of legal actions.

Chapter III

Critical Elements for Microbiology

1. Personnel

1.1 Supervisor/Consultant

The supervisor of the microbiology laboratory must have a bachelor's degree in microbiology, biology, or equivalent. Supervisors who have a degree in a subject other than microbiology must have had at least one college-level microbiology laboratory course in which environmental microbiology was covered. In addition, the supervisor must have a minimum of two weeks training at a Federal agency, State agency, or academic institution in microbiological analysis of drinking water or, 80 hours of on-the-job training in water microbiology at a certified laboratory, or other training acceptable to the State.

If a supervisor is not available, a consultant having the same qualifications may be substituted, as long as the laboratory can document that the consultant is acceptable to the State and is present on-site frequently enough to satisfactorily perform a supervisor's duties.

The laboratory supervisor has the responsibility to insure that all laboratory personnel have demonstrated their ability to satisfactorily perform the analyses to which they are assigned and that all data reported by the laboratory meet the required quality assurance and regulatory criteria.

1.2 Analyst (or equivalent job title)

The analyst must perform microbiological tests with minimal supervision, and have at least a high school education. In addition, the analyst must have a minimum of at least three months of bench experience in water, milk, or food microbiology. The analyst must also have training acceptable to the State, in microbiological analysis of drinking water and a minimum of 30 days of on-the-job training under an experienced analyst. Analysts must take advantage of workshops and training programs that may be available from State regulatory agencies and professional societies. Before analyzing compliance samples, the analyst must demonstrate acceptable results for precision, specificity and satisfactory analysis on unknown samples. Analysts must be closely supervised for at least 6 months after training is completed.

1.3 Personnel Records

Personnel records which include academic background, specialized training courses completed and types of microbiological analyses conducted, must be maintained on laboratory analysts.

1.4 Waiver of Academic Training Requirement

On a case-by-case basis, the certification authority may waive the need for the above specified academic training for supervisors of drinking water system laboratories that only analyze samples from that system. If such a waiver is granted for the supervisor, the certification authority will prepare a written and signed justification for such a waiver and have it available for inspection. Laboratories should keep a copy of the waiver.

The certification authority may also waive the above specified training requirement, on a case-by-case basis, for highly experienced analysts.

2. Laboratory Facilities

Laboratory facilities must be clean, temperature and humidity controlled, and have adequate lighting at bench tops. They must have provisions for disposal of microbiological waste.

Laboratory facilities must have sufficient bench-top area for processing samples; storage space for media, glassware, and portable equipment; floor space for stationary equipment (incubators, water baths, refrigerators, etc.); and associated area(s) for cleaning glassware and sterilizing materials.

3. Laboratory Equipment and Supplies

The laboratory must have all the equipment and supplies needed to perform the approved methods for which certification has been requested. Note: ***Quality control items, designated by QC must be properly documented.***

3.1 pH Meter

Accuracy and scale graduations must be within ± 0.1 units. pH buffer aliquots should be used only once. Electrodes should be maintained according to the manufacturer's recommendations.

QC Record pH meter slope monthly after calibration. Some meters give an automatic slope read-out. The formula for calculating the slope for pH meters that do not have this feature is:

$$\text{Slope (\%)} = |\text{mV at pH 7} - \text{mV at pH 4}| \times 100/177 \qquad (\text{mV} = \text{millivolts})$$

If the slope is <95% or >105%, the electrode or meter may need maintenance.

QC pH meters must be standardized before each use period with pH 7.0 and either pH 4.0 or 10.0 standard buffers, whichever range covers the desired pH of the media or reagent. The date and buffers used must be recorded in a log.

QC Commercial buffer solution containers must be dated upon receipt and when opened. Buffers must be discarded by the expiration date.

3.2 *Balance (top loader or pan)*

Balances should have a readability of 0.1 g. Balances should provide a sensitivity of at least 0.1 g for a load of 150 g and 1 mg for a load of 10 g or less.

QC Balances must be calibrated monthly using ASTM type 1, 2, or 3 weights (minimum of three traceable weights which bracket laboratory weighing needs). Calibration records which include correction factor and tech initials should be on file and used.

QC Non-reference weights must be calibrated every six months with reference weights.

QC Reference weights must be re-certified every five years, damaged or corroded weights replaced.

QC Annual service by a qualified independent technician should be performed. Service and maintenance records should be available.

3.3 *Temperature Monitoring Device*

Glass/mercury or electronic thermometers must be graduated in 0.5°C increments or less, (0.2°C increments or less for tests incubated at 44.5°C) except as noted for hot air ovens (10°C or less) and refrigerators (1°C).

There must be no separation in the mercury or fluid column of glass thermometers.

Dial thermometer that cannot be calibrated should not be used.

QC Glass/electronic thermometers must be calibrated annually and dial thermometers calibrated quarterly at the temperature used against a NIST traceable reference thermometer or one meeting the requirements of NBS Monograph SP 250-23.

QC Records should include date of calibration, identification number (or serial#) of each thermometer, serial # of the NIST thermometer, temperature of each thermometer and the NIST reference thermometer, correction (or calibration) factor and the analysts initials. Thermometers should be tagged with the ID number, correction factor, and the date of calibration and should be discarded if off more than 1°C from the reference thermometer.

QC The adjusted temperature should be recorded.

QC The NIST reference thermometer should be calibrated by a professional at least every 5 years.

Reference thermometer calibration documentation should be maintained.

QC Continuous recording devices that are used to monitor incubator temperature must be recalibrated at least annually using the NIST reference thermometer.

3.4 *Incubator Unit and Water Bath*

Incubator units must have an internal temperature monitoring device and maintain a temperature of 35 ± 0.5°C, and if used, a 44.5 ± 0.2°C water bath. Non-portable incubators should have thermometers placed on the top and bottom shelves of the use area with the thermometer bulb immersed in liquid (except electronic thermometers). Water bath thermometers must be properly immersed. Laboratories which use the enzyme substrate tests with air-type incubators

should note the procedural consideration mentioned in 5.6. An incubation temperature of 44.5 ± 0.2°C for water baths can be best maintained with a gable cover and a pump to circulate water. In Dri-bath incubators, the required temperature must be maintained in all tube wells.

QC Calibration-corrected temperature must be recorded for days the incubator is in use at least twice per day with readings separated by at least 4 hours. Logs should include date and time of reading, temperature and tech initials.

3.5 Autoclave

The autoclave should have an internal heat source, a temperature gauge with a sensor on the exhaust, a pressure gauge, and an operational safety valve. It should maintain the sterilization temperature during the sterilizing cycle and complete an entire cycle (start to finish) within 45 minutes when a 12-15 minute sterilization period is used. The autoclave should depressurize slowly enough to ensure that media will not boil over and bubbles will not form in inverted tubes. Because of safety concerns and difficulties with operational control, pressure cookers must not be used. Autoclave door seals must be clean, no spilt caramelized media, drain screens clean and free of debris

QC The date, contents, time-in, time at sterilization temperature, time-out, total time for each cycle, temperature attained, and analyst's initials must be recorded each time the autoclave is used.

QC Service maintenance must be conducted at least annually and copy of the service contract or internal maintenance protocol and maintenance records must be kept and available for inspection.

QC A maximum-temperature-registering thermometer or continuous recording device must be used during each autoclave cycle to ensure that the proper temperature was reached, and the temperature recorded. Overcrowding must be avoided to allow steam to effectively surround each item being sterilized.

QC Spore strips or ampoules must be used monthly to confirm sterilization. Chemical indicators do not necessarily confirm sterilization.

QC Automatic timing mechanisms must be checked quarterly with a stopwatch or other accurate timepiece or time signal.

3.6 Hot Air Oven

The oven must maintain a stable sterilization temperature of 170-180°C for at least two hours. Only dry items must be sterilized and overcrowding avoided. The oven thermometer must be graduated in 10°C increments or less, with the bulb placed in sand during use.

QC The date, contents, sterilization time and temperature of each cycle, and analyst's initials must be recorded.

QC Spore strip must be used monthly to confirm sterilization. (Avoid spore ampules that might explode or melt.)

3.7 Colony Counter

A dark field colony counter must be used to count colonies for the Heterotrophic Plate Count. Dust or disinfect as needed.

3.8 Conductivity Meter

Meters must be suitable for checking laboratory reagent-grade water and readable in appropriate units (micromhos or microsiemens per centimeter). Use an instrument capable of measuring conductivity with an error not exceeding 1% or 1 micromho per centimeter, whichever is more lenient. If an in-line unit cannot be calibrated, it must not be used to check reagent-grade water.

QC Calibrate the meter at least monthly following the manufacturer's recommendation using a certified and traceable low level standard. If the meter cannot be calibrated with a commercial standard, the cell constant must be determined monthly using a method indicated in Section 2510, "Conductivity," in *Standard Methods.*

3.9 Refrigerator

Refrigerator must maintain a temperature of 1-5°C. Thermometer must be graduated in at least 1°C increments, with the bulb immersed in liquid.

QC The temperature must be recorded for days in use at least once per day.

3.10 ***Inoculating Equipment***

Sterile metal or disposable plastic loops, wood applicator sticks, sterile swabs, or sterile plastic disposable pipet tips may be used. Wood applicator sticks should be sterilized by dry heat. Metal inoculating loops and/or needles should be made of nickel alloy or platinum. (Do not use nickel alloy loop for oxidase test.)

3.11 ***Membrane Filtration Equipment*** *(if used)*

MF units must be stainless steel, glass, or autoclavable plastic, leakproof, not scratched or corroded. A 10X to 15X stereo microscope with a fluorescent light source must be used to count colonies. Membrane filters must be certified for total coliform water analysis. Manufacturer approval is based on data from tests for toxicity, recovery, retention, and absence of growth-promoting substances. Filters must be cellulose ester, white, gridmarked, 47-mm diameter, and 0.45 µm pore size, or alternate pore sizes if the manufacturer provides performance data equal to or better than the 0.45 µm pore size. Membrane filters must be purchased presterilized or autoclaved for 10 minutes at 121°C before use. Ensure that filters are not brittle or distorted and manufacturer's specification or certification sheet is available. Forceps used for picking the membrane should be blunt and smooth-tipped.

QC If graduated funnels are used to measure sample volume, accuracy must be checked with a Class B graduated glassware or better. Tolerance must be ≤2.5%.

QC The lot number for membrane filters and the date received must be recorded. Each lot of commercially prepared and each batch of laboratory prepared membrane filters must be checked for sterility before use.

3.12 ***Culture Dishes (loose or tight lids)***

Presterilized plastic or sterilizable glass culture dishes should be used. Maintain sterility of reusable culture dishes by placing in stainless steel or aluminum canisters, or wrap with heavy aluminum foil or char-resistant paper. Loose-lid petri dishes should be incubated in a tight-fitting container, e.g., plastic vegetable crisper containing a moistened paper towel to prevent dehydration of membrane filter and medium. Opened packs of disposable culture dishes should be resealed between use periods.

3.13 ***Pipettes***

To sterilize and maintain sterility of glass pipettes, stainless steel or aluminum canisters should be used, or individual pipettes wrapped in char-resistant paper or aluminum foil. Pipettes should have legible markings and must not be chipped or etched. Opened packs of disposable sterile pipettes should be resealed between uses. Pipette tips for calibrated micropipetters should be sterile.

QC Reusable pipettes delivering volumes of 10 ml or less must be accurate within a 2.5% tolerance.

QC Micropipetters must be calibrated annually and adjusted or replaced if the accuracy is outside 2.5%.

3.14 ***Glassware and Plasticware***

Glassware must be made of borosilicate glass or other corrosion-resistant glass with no chips or cracks. Markings should be legible. Plastic items must be clear and non-toxic to microorganisms. Culture tubes and containers should be of sufficient size to contain medium plus sample without being more than three-quarters full. Tube closures should be stainless steel, plastic, aluminum, or screw caps with non-toxic liners. Cotton plugs should not be used.

QC Graduated cylinders for measuring sample volume must be accurate to within a 2.5% tolerance. (Use Class A previously verified graduated cylinder for calibrating or else use clearly marked precalibrated containers, e.g. flasks, beakers).

3.15 ***Sample Containers***

Sample containers should be wide-mouth plastic or non-corrosive glass bottles with non-leaking ground glass stoppers or caps with non-toxic liners that must withstand repeated sterilization. Leakproof sterile plastic bags containing sodium thiosulfate may also be used. The capacity of sample containers should be at least 120 mL (4 oz.). Reusable glass stoppers must be covered with aluminum foil or char-resistant paper for sterilization. Nonsterile containers should be sterilized in autoclave or in hot air oven (glass containers). Sample containers should be moistened with several drops of water or adequate amount of sodium thiosulfate (0.1 ml 10% $Na_2S_2O_3$) solution before autoclaving to prevent an "air lock" sterilization failure.

QC At least one sample container must be selected at random from each batch of laboratory prepared and each lot of commercially prepared sterile sample bottles for sterility check. (Add approximately 25 mL of a sterile non-selective broth, e.g., tryptic soy, trypticase soy, or tryptone broth incubate at 35 ± 0.5°C for 24 hours and checked for growth after 24 and 48 hours.) Re-sterilize the entire batch if growth is detected.

3.16 ***Ultraviolet lamp (254 nm Germicidal unit)*** (if used)

The 254-nm germicidal unit used for sanitizing MF equipment should be disconnected monthly and the lamps cleaned by wiping with a soft cloth moistened with ethanol. The 365-366-nm longwave unit used for fluorometric tests should also be maintained the same way.

QC The UV lamp (254 nm) used for sanitizing should be tested quarterly with an UV light meter. The lamp should be replaced if it emits less than 70% of its initial output. Using an agar spread plate containing 200 to 250 microorganisms can also test germicidal effectiveness. The culture is exposed to the UV light for two minutes and should show a count reduction of 99%. If not, the lamp should be replaced. Other methods may be used if results demonstrate the same effectiveness. Protective eyewear should be used when checking the 254-nm lamp.

3.17 ***Spectrophotometer or Colorimeter*** (if used)

Wavelengths should be in the visible range-Spectronic 20 (ThermoSpectronic), or equivalent, with cell holder for ½ inch diameter cuvettes (Model# 4015) or 13mm x 100mm cuvettes.

QC A calibration standard and a method-specific blank should be analyzed everyday the instrument is used, prior to sample analysis. The commercially obtained calibration standard should give a reading in the desired absorbance range.

3.18 ***MPN (Quanti-Tray) Tray Sealer*** (if used)

A regular cleaning schedule should be maintained by disinfecting the surfaces and other removable parts. The spill basin should also be emptied periodically.

QC The sealer should be checked monthly by adding dye to a water sample. If dye is observed outside the wells, perform maintenance and/or check technique.

4. General Laboratory Practices

Although safety criteria are not covered in the laboratory certification program, laboratory personnel should be aware of general and customary safety practices for laboratories. Each laboratory is encouraged to have a safety plan available and follow the personal protective guidelines provided in the material safety data sheets accompanying the receipt of toxic materials or reagents.

4.1 ***Sterilization Procedures***

The required times for autoclaving certain items at 121°C are listed below. Except for membrane filters and pads and carbohydrate-containing media, indicated times are minimum times which may necessitate adjustment depending upon volumes, containers, and loads. Carbohydrate-based media should not be over-sterilized.

Table 4:1 Sterilization Times

Item	Time (min)
Membrane filters & pads	10*
Carbohydrate containing media	12-15*
Contaminated test materials	30
Membrane filter assemblies	15**
Sample collection bottles	15
Individual glassware	15
Dilution water blank	15
Rinse water (0.5 - 1 L)	15-30***

* Autoclaved membrane filters and pads and all media must be removed immediately after completion of the sterilization cycle

** Membrane filter equipment must be autoclaved before the beginning of the first filtration series. A filtration series ends when 30 minutes or longer elapses after a sample is filtered.

*** Time depends upon water volume per container and autoclave load

Ultraviolet light (254 nm) may be used for sanitizing MF equipment to reduce bacterial carry-over between samples during a filtration series.

4.2 *Reagent-Grade Water*

4.2.1 Only satisfactorily tested reagent water from stills or deionization units may be used to prepare media, reagents, and dilution/rinse water for performing bacteriological analyses.

QC The suitability test of the reagent water should be done and meet the following criteria:

Table 4:2 Reagent Water Quality Criteria

Parameter	Limits	Frequency
Conductivity	<2 micromhos/cm (microsiemens/cm) at 25°C or >0.5 megohms resistance	Monthly4
Total Chlorine Residual[1]	<0.1 mg/L	Monthly
Heterotrophic Plate Count[2]	< 500 CFU/ml	Monthly
Pb, Cd, Cr, Cu, Ni, Zn	Not greater than 0.05 mg/L per contaminant. Collectively, no greater than 0.1 mg/L	Annually
Bacteriological Quality of Reagent Water[3]	Ratio of growth rate 0.8 - 3.0	Annually

[1] DPD Method should be used. Not required if source water is not chlorinated.

[2] Pour Plate Method. See Standard Methods 9215B.

[3] See Standard Methods, Section 9020B. This bacteriological quality test is not needed for Type II water or better or Medium quality reagent water or better, as defined in Standard Methods, Section 1080C. If these types of water are not available, and a glass still is used for reagent water, a silicon test that meets the specifications described in SM, Section 1080C should be used.

4 Monthly, if meter is in-line or has a resistivity indicator light; otherwise, with each new batch of reagent water

4.2.2 Laboratories that purchase their reagent-grade water should follow these guidelines for water quality testing requirements:

QC Test each new lot of water for sterility and autofluorescence (if applicable) and every 6 months if not used entirely. Keep records on file for each lot.

QC Maintain the manufacturer's certificates of analysis and specifications on file.

4.3 Dilution/Rinse Water

Stock buffer solution or peptone water should be prepared, as specified in Standard Methods, Section 9050C. Stock buffers should be autoclaved or filter-sterilized, the containers labeled and dated. It should be refrigerated and not turbid.

QC Each batch (or lot if commercially prepared) of dilution/rinse water should be checked for sterility by adding 50 ml of water to 50 ml of a double strength non-selective broth (e.g., tryptic soy, trypticase soy or tryptose broth). Incubate at 35 ± 0.5°C and check for growth after 24 and 48 hours. Discard if growth is detected.

4.4 Glassware Washing

Laboratory glassware must be washed with a detergent designed for laboratory use. Distilled or deionized water should be used for final rinse.

QC A glassware inhibitory residue test (Standard Methods, Section 9020B) must be performed before the initial use of a washing compound and whenever a different formulation of washing compound, or washing procedure, is used.

QC Batches of dry glassware must be spot-checked for pH reaction, especially if glassware is soaked in alkali or acid (Standard Methods, Section 9020B). Use 0.04% bromthymol blue (or equivalent pH indicator) and observe color reaction (blue-green for btb). These tests will ensure that glassware is at a neutral pH and is free of toxic residue.

5. Analytical Methodology

- Laboratories must use only the analytical methodology specified in the Total Coliform Rule (40 CFR 141.21(f)) and the Surface Water Treatment Rule (40 CFR 141.7 (a)) and the Groundwater Rule. See Appendix D. A laboratory must be certified for all analytical methods (see Appendix B) that it uses for compliance purposes. At a minimum, the laboratory must be certified for one total coliform method and one fecal coliform or *E. coli* method. A laboratory should also be certified for a second total coliform method if one method cannot be used for certain drinking waters (e.g., where the water usually produces confluent growth on a plate). Laboratories that enumerate heterotrophic bacteria (HPC) for compliance with SWTR must be certified either for the Pour Plate Method or the Simplate method.

- Before analysis, water samples must be shaken vigorously (about 25 times). The required volume for the analysis of Total coliforms in drinking water must be 100 ± 2.5 ml.

- Use of dehydrated or commercially prepared media is recommended. Dehydrated media must be stored in a cool, dry location. Media should be discarded if it becomes discolored or caked, and definitely by the manufacturer's expiration date. Ampouled media discarded by manufacturer's expiration date.

- Prepared plates may be refrigerated in sealed plastic bags or containers. Each bag or container should include the date of preparation and the date of expiration. Refrigerated media should be brought to room temperature before use. Media with growth or bubbles should be discarded. Liquid media should be discarded if greater than 10% of the volume has evaporated. The following table shows the storage criteria for laboratory prepared media:

Table 5:1 Laboratory Prepared Media Storage Criteria

Laboratory prepared media	Storage temp.	Max. storage time
Poured agar plates	1 – 5 °C	2 weeks
Broth in tubes, bottles or flasks with loose fitting closures	1 – 30 °C	2 weeks
Broth in tightly closed screw-cap tubes, bottles or flasks	1 – 30 °C	3 months

QC If media is stored at <25°C a daily temperature reading must be taken in the area of media storage. Media must be

colonies or 20 – 60 fecal coliform colonies.

- Funnels should be rinsed 2-3 times after each sample filtration with 20-30 ml portions of sterile rinse water and again, 2-3 times after the filters are removed. Instead of the rinses, the funnel can be sanitized by exposing it to a 254-nm UV light for 2 minutes to prevent carry-over between samples, especially for surface water samples.
- Absorbent pads must be saturated with the broth medium (at least 2 ml) with the excess decanted.

QC At least one MF and filtration unit sterility check should be conducted at the beginning and the end of each filtration series by filtering 20-30 ml of dilution water. If the control indicates contamination, all data from affected samples must be rejected and an immediate resampling should be requested. A filtration series ends when 30 minutes or more elapse between sample filtration.

5.1.1 Media

Prepared medium should be refrigerated. Bring to room temperature before use. Plates with broth medium must be discarded after two weeks. Commercially prepared media ampules should be discarded according to the manufacturer's expiration date. Broth, plates or ampoules should be discarded earlier if contamination is observed. See 5.1 for items to be included in the media preparation records.

5.1.1.1 **M-Endo Medium** broth or agar (also known as M-Endo broth MF and M-Coliform Broth) or **LES Endo agar** (also known as M-Endo Agar LES) for detecting Total coliforms in drinking water or enumerating Total coliforms in source water: Medium must be used in the single step or enrichment techniques. Ensure that ethanol used in the rehydration procedure is not denatured. Prepare medium in a sterile flask in a boiling water bath or on a hot plate with a stir bar to bring the medium just to the boiling point. **The medium must not be boiled.** Final pH must be and 7.2 ± 0.1 for M-Endo medium and 7.2 ± 0.2 for LES Endo agar. [MF broth must be refrigerated no longer than 96 hours, poured MF agar plates no longer than two weeks, and ampoules of M-Endo broth in accordance with the manufacturer's expiration date.] Media must be discarded earlier if contamination is observed.]

5.1.1.2 **m-ColiBlue24 medium** for detecting Total coliforms and *E. coli* in drinking water: Mix broth by inverting ampoules 2-3 times before breaking. Contents should be poured evenly over absorbent pad. Refrigerate unopened ampoules until the expiration date. Discard if growth is detected. The final pH must be 7.0 ± 0.2.

5.1.1.3 **MI Medium** (with or without agar) for detecting Total coliforms and E. coli in drinking water or enumerating Total coliforms in source water: The commercially prepared, presterilized medium contains the antibiotic, cefsulodin and should not be autoclaved or overheated. Melt bottled agar in a boiling water bath (or per manufacturer's recommendation). As soon as agar melts completely, cool slightly and pour immediately into sterile plates. Excessive heat will destroy the effectiveness of the antibiotic. Dehydrated medium should be prepared and autoclaved according to the manufacturer's instructions. Cool the agar, add freshly prepared, filter-sterilized cefsulodin and pour immediately into sterile plates. The final pH of MI agar must be 6.95 ± 0.20 and the broth, 7.05 ± 0.20.

5.1.1.4 **Chromocult® Coliform Agar** for detecting Total coliforms and *E. coli* in drinking water: Do not autoclave or overheat. The final pH must be 6.8 ± 0.2. If a heavy background of heterotrophic bacteria is expected, especially Pseudomonas and Aeromonas spp., add cefsulodin solution to the cooled medium. Dissolve 10 mg cefsulodin in 2 ml deionized or distilled water, then add this solution to 1 liter of medium.[®]

5.1.1.5 **Coliscan®** for detecting Total coliforms and E. coli in drinking water or enumerating Total coliforms in source water: Coliscan is available in dry powder agar or already pre-sterilized bottled agar. For reconstitution and antibiotic addition, follow the manufacturer's protocol (Micrology Laboratories, LLC). The final pH must be 7.00 ± 0.20.

5.1.1.6 **m-FC Broth** (with or without agar) for enumerating fecal coliforms in source water: Do not autoclave. Bring medium just to the boiling point. The final pH must be 7.4 ± 0.2.

[®] EMD Chemicals, Inc., www.emdchemicals.com, (800) 222-0342

protected against light exposure and extreme temperatures.

QC Records for laboratory prepared media should include the date of preparation, media manufacturer, type of medium, lot number, time-in, time at sterilization temperature, time-out, total time autoclaved or boiled, temperature attained, positive and negative growth control results, sterility check results, final pH, date of expiration, and the technician's initials. The rack/basket/bag for each batch of laboratory prepared media is labeled with media type, date of preparation, and date of expiration.

QC Records for commercially prepared liquid media should include the media manufacturer, lot number, type of media, date received, final pH verification, positive and negative growth control results, sterility check, date of expiration, and recording technicians initials. Medium must be discarded by manufacturer's expiration date. Containers/boxes/bags of commercially prepared media must be identified with the media type, lot number, date of receipt, date opened, and date of expiration.

QC Each new lot of dehydrated or prepared commercial medium and each batch of laboratory prepared medium should be checked before use for sterility and with positive and negative culture controls. Control organisms can be stock cultures, periodically checked for purity, or commercially available disks impregnated with the organism. Those laboratories using commercially prepared media with manufacturer shelf lives of greater than 90 days should run positive and negative controls each quarter in addition to the above. Frequent positive and negative control checks are recommended.

Table 5: 2 Recommended quality control organisms:

Analytes	Positive Control	Negative Control
Total coliforms	*Escherichia coli* *Enterobacter aerogenes*	*Staphylococcus aureus* *Proteus vulgaris* *Pseudomonas aeruginosa*
Fecal coliforms	*Escherichia coli* *Klebsiella pneumoniae* (thermotolerant)	*Enterobacter aerogenes*
Escherichia coli	*Escherichia coli* (MUG-positive strain)	*Enterobacter aerogenes* *Klebsiella pneumoniae* (thermotolerant, MUG-negative)
Enterococci	*Enterococcus faecalis* *Enterococcus faecium*	*S. aureus* *E. coli* *Serratia marcesens* (MUG-negative)

QC Laboratories should perform parallel testing between a newly approved test and another EPA-approved procedure for enumerating total coliforms for at least several months and/or over several seasons to assess the effectiveness of the new test for the wide variety of water types submitted for analysis. During this testing, spiking the samples occasionally with sewage or a pure culture may be necessary to ensure that some of the tests are positive. See Appendix C for a list of approved analytical methods, applicable regulations and cited SM methods.

To do the comparison study, perform at least 50 parallel tests using the old and the new method, using a variety of samples with varying dilutions or concentrations. 30-50% must be in the positive range. Samples maybe spiked to obtain positive results. After the study, submit the following documents to the Certification Office to be reviewed:

- Summary of the parallel study showing all the results obtained
- SOP for the new method
- All related QC records
- A satisfactory proficiency test (Performance Evaluation) result

Once all requirements are met, an interim certification (if onsite audit is not yet due) will be issued.

5.1 Membrane Filter (MF) Methods

(for detecting Total coliforms and E. coli in drinking water, enumerating Total coliforms or Fecal coliforms in source water, and detecting E. coli in ground water)

- For source water samples under the SWTR, appropriate dilutions must be used to yield 20 – 80 Total coliform

5.1.2 The following table shows the ***incubation period and colony appearance*** for each of the media described above:

Table 5:3 Membrane Filtration Media Incubation and Colony Appearance

Medium	Sample*	Incubation	Total coliform colony	*E. coli* colony
M-Endo	DW/SW	35 ± 0.5 °C for 22-24 hours	Metallic (golden) sheen - presumptive	N/A
m-ColiBlue24	DW	35 ± 0.5 °C for 24 hours	Red	Blue to purple
MI	DW/SW	35 ± 0.5 °C for 22-26 hours	Fluorescent under UV light	Blue under normal light
Chromocult	DW	36 ± 1 °C for 23-25 hour	Salmon to red	Dark-blue to violet**
m-FC	SW	44.5 ± 0.2 °C for 22-26 hours	N/A	Blue (Fecal coliforms)
Coliscan	DW/SW	32-37 °C for 24-28 hours	Pink-magenta	Purple-blue

* DW (Drinking Water) / SW (Source Water)

**Add one drop of Kovac's reagent to each dark-blue to violet colony. The formation of a cherry-red color within seconds confirms the presence of *E. coli.*

5.1.3 **Invalidation of Total coliform-negative drinking water sample: Samples** resulting in confluent or TNTC (too numerous count) growth must be invalidated unless at least one coliform-type colony is detected. (See Table 5.2.3 for colony appearance of Total coliform or *E. coli* on various media). If no total coliforms are detected, record the result as "confluent growth" or "TNTC" and request an additional sample from the same sampling site. Confluent growth is defined as a continuous bacterial growth covering the entire membrane filter and TNTC is defined as greater than 200 colonies on the membrane filter in the absence of detectable coliforms. However, before invalidation, the laboratory may perform a verification test for Total coliform and fecal coliform/*E. coli* on the confluent or TNTC growth. If the verification test is Total coliform-positive, the sample must be reported as such. If the test is Total coliform-negative, the sample must be invalidated. (A fecal coliform/*E. coli*-positive result is considered a total coliform-positive, fecal coliform/*E.coli*-positive sample, even if the initial and/or verification total coliform test is negative.)

5.1.4 **Invalidation of source water samples (SWTR):** Any samples which results in confluent growth or TNTC must be invalidated even when Total coliform or Fecal coliform colonies are present because coliform density must be determined.

5.1.5 **For drinking water samples:** ***Total*** coliform-positive colonies must be tested for fecal coliforms or *E. coli.* When EC Medium or EC + MUG medium is used, the colonies must be transferred using an approved method. If a single swab is used to wipe the entire surface of the presumptive Total coliform-positive MF culture to inoculate three different media, then inoculate the media according to the following order: 1st EC or EC + MUG medium, 2nd lauryl tryptose broth (LTB), 3rd 2% brilliant green lactose bile broth (BGLBB). To verify colonies on M-Endo medium, all sheen colonies (pick all sheen colonies up to a maximum of five) must be verified using either single strength lactose broth (LB) or LTB and then single strength 2% BGLBB. The EPA-approved cytochrome oxidase and ß-galactosidase rapid test procedure may also be used for verification. Individual colonies can be transferred with a sterile needle, loop, or applicator stick. If no sheen colonies are observed, verify up to five red questionable colonies. When using single inoculum for different verification media, always inoculate BGLBB last. For Nutrient Agar + MUG Test, refer to Section 5.8.

5.1.6 **For source water samples (SWTR):** ***Initial*** Total coliform counts must be adjusted based upon verified data.

QC A monthly accuracy check must be performed by having 2 or more analyst count total or fecal coliform colonies from the same membrane. The counts must agree within 10%.of each other. (for source water samples)

5.2 *Multiple Tube Fermentation Technique (MTF) or Most Probable Number (MPN)*

(for detecting Total coliforms in drinking water and enumerating Total coliforms in source water)

For drinking water samples, various testing configurations can be used (CFR141.21 (f)(3), as long as a total sample volume of 100 mL is examined for each test. For source water samples, at least 3 series of 5 tubes each with appropriate sample dilutions must be used (e.g., 0.1 ml, 0.01 ml, and 0.001 ml).

5.2.1 Media

Lauryl tryptose broth (LTB), also known as lauryl sulfate broth (LST) must be used in the presumptive test and 2% BGLBB in the confirmed test. If lactose broth (LB) is used, a parallel study of at least 25 samples must be conducted with LTB. The comparison must show less than 10% false-positives and false-negatives. The study should be documented and records retained.

The test medium concentration must be adjusted so that even with the addition of the sample, the media concentration remains single strength. Refer to SM 9221B Table 9221:I for LTB media adjusted preparation. If the inverted vial is omitted and a single 100-mL sample volume is used, an acid indicator (0.01 g/L bromcresol purple) can be added to the medium to determine acid production and prevent problems associated with gas bubbles in large inverted tubes. The media must be autoclaved at 121°C for 12-15 minutes. The final pH must be 6.8 ± 0.2 for LTB and 7.2 ± 0.2 for BGLBB.

Sterile medium in tubes must be examined to ensure that the inverted vials, if used, are free of air bubbles and are at least one-half to two-thirds covered after the water sample is added.

If MTF media are refrigerated after sterilization, they must be incubated overnight at room temperature before use. Tubes/bottles showing growth and/or bubbles must be discarded. If prepared broth media are stored, they must be maintained in the dark at <25°C no longer than three months for screw-cap tubes/bottles and two weeks for tubes/bottles with loose-fitting closures. Media must be discarded if evaporation exceeds 10% of the original volume.

5.2.2 Incubation

After the medium is inoculated, it must be incubated at 35 ± 0.5°C for 24 ± 2 hours. If no gas or acid is detected, it must be incubated for another 24 hours (total incubation time 48± 3 hours).

5.2.3 Results

All samples that produce a turbid culture (i.e., heavy growth, and opaque) in the absence of gas/acid production, in LTB, must be invalidated. (See 5.3.5 below) Each 24- and 48-hour gas-positive or acid-positive tube must be confirmed with 2% BGLBB. A completed test is not required. For drinking water samples, Total coliform-positive samples must be tested for the presence of fecal coliforms or E. coli.

5.2.4 Invalidation of Total coliform-negative samples (drinking water samples)

All samples that produce a turbid culture (i.e. heavy growth) in the absence of gas/acid production, in LTB or LB, must be invalidated. The Total Coliform Rule requires that laboratories invalidate presumptive tubes with heavy growth without gas production and request that the system provide another water sample from the same site within 24 hours. Before invalidation, the laboratory may perform a confirmed test (and/or a fecal coliform/ *E. coli* test) on the total coliform-negative culture to check for coliform suppression. If the confirmed test is Total coliform-positive ((or Fecal coliform/*E. coli* positive), the laboratory may consider the first sample as Total coliform-positive* and the sample reported as such. If the confirmation test is total coliform-negative, the sample must be **invalidated** because high levels of non-coliform bacteria in the presumptive tubes may have killed or suppressed the growth of the coliforms.

5.2.5 Invalidation of Total coliform-negative samples (source water samples)

All samples that produce a turbid culture (i.e. heavy growth) in the absence of gas/acid production, in LTB or LB, must be invalidated. The laboratory must collect, or request that the system collect another sample within 24 hours from the same site. Before invalidation, the laboratory may perform a confirmed test on the total coliform-negative culture. If the confirmed test is Total coliform-positive, the MPN should be reported. If the test is Total coliform-negative, the sample should be invalidated.

5.3 Presence-Absence (P-A) Coliform Test *(for detecting Total coliforms in drinking water)*

5.3.1 Medium

The medium must be autoclaved for 12 minutes at 121°C. Total time in the autoclave must be less than 30 minutes. Space should be allowed between bottles. The final pH must be 6.8 ± 0.2. Six-time formulation strength may be used and if used, the medium must be filter-sterilized rather than autoclaved. If prepared medium is stored, it must be maintained in a culture bottle at <30°C in the dark for no longer than three months. If evaporation exceeds 10% of original volume, the medium must be discarded.

5.3.2 Procedure

A 100-mL sample must be inoculated into a P-A culture bottle, incubated at 35 ± 0.5°C and observed for a yellow color (acid) after 24 and 48 hours. Yellow cultures must be confirmed in BGLBB and a fecal coliform/*E.coli* test conducted.

5.3.3 Invalidation

All samples that produce a non-yellow turbid culture must be invalidated. The laboratory must collect, or request that the system collect, another sample from the same site. Before invalidation, the laboratory may perform a confirmed test (and/or a Fecal coliform / *E coli* test) on the presumptive Total coliform-negative culture. If the confirmed test is total coliform-positive, the sample must be reported as such. If the confirmed test is negative, the sample must be invalidated. A fecal coliform/*E. coli* positive result is considered a total coliform-positive, fecal coliform/*E. coli* positive sample, even if the presumptive and/or confirmed total coliform test is negative.

5.4 Fecal Coliform Test *(using **EC Medium** for Fecal coliforms in drinking water or source water), (using **A-1 Medium** for Fecal coliforms in source water only)*

5.4.1 EC Medium

- This medium is used to determine whether a total coliform-positive culture taken from the distribution system contains fecal coliforms, in accordance with the Total Coliform Rule. The laboratory must transfer each total coliform-positive culture from a presumptive tube/bottle, or each presumptive total coliform-positive colony (minimum of five colonies), to at least one tube containing EC medium with an inverted vial.
- EC Medium may also be used to enumerate fecal coliforms in source water in accordance with the Surface Water Treatment Rule. Perform the presumptive phase of the MTF test first. Three sample volumes of the source water (e.g. 10, 1 and 0.1 ml) 5 or 10 tubes/sample volume should be used. Each positive presumptive culture must be transferred to a tube containing EC medium.
- EC Medium must be autoclaved for 12-15 minutes at 121°C. The final pH must be 6.9 ± 0.2. Inverted vials must be examined to ensure that they are free of air bubbles. The inverted vial must be at least one-half to two-thirds covered. If prepared medium is stored, it must be maintained in the dark at <30°C. Prepared medium stored in tubes with loose-fitting closures must be used within two weeks. Prepared medium stored in tightly closed screw type tubes may be kept up to three months. (See Table 5.1) If the medium is stored in a refrigerator, it must be incubated overnight at room temperature before use; tubes that show growth and/or bubbles must be discarded. After inoculation, EC medium must be incubated at 44.5 ± 0.2°C for 24 ± 2 hours making sure that the water level of the water bath adequately immerses the culture tubes. Any amount of gas detected in the inverted vial of a tube that has turbid growth is considered a fecal coliform-positive test.

5.4.2 A-1 Medium

- A-1 Medium may be used as an alternative to EC Medium to enumerate fecal coliforms in source water, in accordance with the Surface Water Treatment Rule. A-1 Medium must not be used for drinking water samples. Three volumes of source water (e.g., 10, 1 and 0.1 ml) 5 or 10-tubes/sample volume should be used. Unlike EC medium, A01 Medium may be used for direct isolation of fecal coliforms from water.
- A-1 Medium must be sterilized by autoclave at 121°C for 10 minutes. The final pH must be 6.9 ± 0.1. Inverted vials should be free of air bubbles. (See Table 5.1 for storage criteria). Inoculated medium is incubated at 35 ± 0.5°C for three hours then at 44.5 ± 0.2°C for 19-23 hours. Any amount of gas detected in the inverted vial of a tube that has turbid growth is considered a fecal coliform-positive test regardless of the results of any subsequent test on the presumptive culture.

[Proposed]

5.4.3 **Azide Dextrose Medium Test** *(for detecting fecal streptococci in ground water)*

For testing 100-ml samples, prepare triple strength (3X) formulation in a culture bottle and autoclave at 121°C for 15 minutes. Final pH must be 7.2 ± 0.2. Add 100-ml sample to the medium and incubate at 35 ± 0.5°C. Check for turbidity after 24 ± 2 hours. If there is no turbidity, re-incubate and check again after a total incubation period of 48 ± 3 hours. A turbid culture may be confirmed as fecal streptococci by streaking a portion of the broth onto bile esculin agar (BEA) or bile esculin azide agar (BEAA). Before streaking, BEA and BEAA must be sterilized by autoclaving at 121 ° C for 15 minutes. Final pH must be 6.6 ± 0.2 for BEA and 7.1 ± 0.2 for BEAA. Inoculated plates are incubated at 35± 0.5 °C for 24 ± 2 hours. Brownish-black colonies on BEA or BEAA with brown halos confirm the presence of fecal streptococci. If required, enterococci test can be performed on one or more fecal streptococci colonies by transferring them to brain heart infusion broth (BHI) supplemented with 6.5% NaCl incubated at 35 ± 0.5 °C for 48 hours. Growth indicates the presence of streptococci.

5.5 Enzyme (Chromogenic/fluorogenic) substrate tests *(for Total coliforms and Escherichia coli in drinking water)*

5.5.1 Media and supplies

The media should not be prepared from basic ingredients. Only commercially available media should be used because of quality assurance and uniformity requirements. Prolonged exposure of these media to light should be avoided. Refrigerated media should be brought to room temperature before adding to the sample.

An ultraviolet lamp (365-366 nm, 6 watt) is used for detecting fluorescence produced by *E. coli* in the sample. Any sample that produces an atypical color change (e.g., black or greenish-black indicating high content of iron or Manganese and hydrogen sulfide; blue flash indicating high level chlorine, etc.) should be invalidated. A re-sample from the same site should be requested. Reference comparators that come with some test media should be discarded by manufacturer's expiration date.

QC Some lots of fluorogenic media have been known to autofluoresce. Therefore, each lot of medium must be checked before use with a 366-nm ultraviolet light with a 6-watt bulb. If the media (plus sterile water sample) exhibit even a faint fluorescence, another lot must be used. If the medium (plus sterile water sample) exhibit a color change even before incubation, another lot of medium must be used. The manufacturer should be notified concerning these problems

QC Performance check for each new lot of medium must be done by inoculating sterile water with known cultures of MUG-positive *E. coli* strain, MUG-negative coliform, and non-coliform controls. See Table 5.2 for suggested control organisms.

QC Sample containers should be checked for fluorescence before use with a 366-nm ultraviolet light source with a 6-watt bulb. If fluorescence is observed before incubation, do not use.

QC Incubators, especially small air-types, may not bring a cold 100-ml sample to the specified temperature for several hours. This may cause a false-negative result because of possibly shortened incubation period. The following table provides instructions for certain media incubated in air-type incubators:

Table 5.6:1 Pre-incubation instructions for some chromogenic/fluorogenic substrate tests

Test	Sample Pre-incubation instructions
Colilert (Presence/Absence)	Specified 24-hour incubation time includes time it takes to bring sample temperature to 35 °C
Colilert Quanti-Tray	Specified 24-hour incubation time includes time it takes to bring sample temperature to 35 °C
Colilert-18 (Presence/Absence)	Pre-warm sample in 35 °C water bathe for 20 minutes or 44.5 °C for 7-10 minutes
Colilert-18 Quanti-Tray	Allow sample to equilibrate to rm temp (20-30 °C) before the 18-hr incubation time
Colisure	Allow sample to equilibrate to room temperature (20-30 °C) before the 24-hour incubation time

Readycult (Presence/Absence) and Fluorocult LMX Broth	Specified 24-hour incubation time includes time it takes to bring sample temperature to 36 ± 1 °C
Colitag	Specified 24-hr incubation time includes the time it takes to bring sample temperature up to 35 ± 0.5 °C

5.5.2 Criteria for specific media

5.5.2.1 Colilert/Colilert-18

- Samples must be incubated at 35 ± 0.5°C for 24 hours. A color change to yellow equal to or greater than the reference comparator indicates the presence of total coliforms and must be reported as a total coliform positive. If the sample is yellow, but lighter than the comparator, it must be incubated for another 4 hours (do not incubate more than 28 hours total). If color is still lighter than the comparator at 28 hours, the sample should be reported as negative. A coliform-positive sample that fluoresces under the UV light indicates the presence of *E. coli*.
- Laboratories that use the Colilert-18 test must incubate for 18 hours (up to 22 hours if sample after 18 hours is lighter than the comparator) with at least the first 20 minutes in a 35 ± 0.5°C water bath.
- Do not incubate the Colilert test in a Whirl-Pak bag.
- For enumerating total coliforms in source water using a 5 or 10-tube configuration, Quanti-Tray or Quanti-Tray 2000, the dilution water should be sterile deionized or distilled water, not buffered water.

QC The Quanti-Tray sealer should be checked monthly by adding a dye to a water sample. Leakage outside the wells should prompt maintenance or use of another sealer. Technique should also be observed.

5.5.2.2 Colisure

- Samples must be incubated at 35 ± 0.5°C for 24 or up to 48 hours. If the sample changes from a yellow to a magenta color, the sample is total coliform positive. A coliforms-positive sample that fluoresces under the UV light is positive for *E. coli*.
- This media may also be used for the Quanti-Tray.

5.5.2.3 E*Colite

- Samples must be incubated at 35 ± 0.5°C for 28 hours. Positive Total coliform result is indicated by a color change from yellow to a blue or blue-green or a blue color in the corners of the bag. If *E. coli* is present, fluorescence is exhibited under a UV light. If no fluorescence is observed, re-incubate for an additional 20 hours (up to 48 hours total incubation) then check for fluorescence. If sample turns red, a faulty seal has allowed the bactericide in the 3rd compartment to leak into the media compartment. In this case, discard the sample and request another sample.

5.5.2.4 Readycult Coliforms 100 Presence-Absence

- Samples must be incubated at 36 ± 1°C for 24±1 hours. Positive total coliform result is indicated by a color change from slight yellow to blue-green. The presence of *E. coli* is indicated by a bright light-blue fluorescence under the UV light. Further confirmation of *E. coli* is done by adding Kovac's indole reagent to the positive sample, which shows the immediate (20 seconds) formation of a red ring on the surface of the sample.

5.5.2.5 Fluorocult LMX Broth

- This media is identical to Readycult, except that it is a dehydrated culture medium in granulated form packed primarity in a 500 g plastic bottle. For testing a 100-ml water sample, suspend 34 g of the media in 1L purified water and boil to dissolve completely. Transfer 100-ml aliquots to 250-ml bottles and autoclave for 15 min at 121 °C. Cool to room temperature, add 100-ml water sample and incubate. Do not add any supplement to the medium.

5.5.2.6 Colitag

- Samples are incubated at 35 ± 0.5°C for 24±2 hours. The presence of coliform is indicated by color change from near colorless to a vibrant yellow. The presence of *E. coli* is indicated by a bright blue fluorescence under a UV (366 nm, 6 watt) light. No other confirmation is necessary.

5.5.3 Other considerations

The enzyme substrate tests must not be used to confirm a presumptive total coliform-positive culture in fermentation broth (LTB, LB, P-A Coliform tests) or membrane filtration methods. The turbidity or high density of non-coliforms in the inoculum may either suppress the coliforms or overload the suppressant reagent system and cause false positive results. Laboratories may use the EC+MUG Test to detect *E. coli* in presumptive MTF, P/A or MF cultures.

5.6 EC + MUG Test *(for E. coli)*

To confirm the presence of *E. coli*, transfer a presumptive total coliform-positive culture (from MTF or MF cultures) to EC + MUG medium using an approved method.

EC + MUG medium is available commercially. For laboratory prepared media, the MUG may be added to EC medium before autoclaving. The final MUG concentration must be 50 µg/ml and the final pH must be 6.9 ± 0.2. The inverted vial may be omitted because gas production is not relevant to the test and may cause confusion on result interpretation. See Table 5.1 for storage of prepared media. The test medium must be properly immersed in the water bath and incubated at 44.5 ± 0.2°C for 24±2 hours and then checked for fluorescence using an ultraviolet lamp (366 nm) with a 6-watt bulb in a darkened room.

QC Run positive and negative controls for each new lot or batch of media.

QC Tubes and autoclaved medium must be checked for fluorescence before use. Use only non-fluorescing equipment or media, otherwise, use controls (MUG-positive *E. coli* and MUG-negative or plain medium) for each batch of analysis.

5.7 [Proposed] ***Enterolert Test*** *(for Enterococci in ground water)*

Medium must be stored in the dark at 4-30 °C until use.
Add Enterolert reagent to 100-ml water sample and incubate at 41 ± 0.5°C for 26±2 hours. Fluorescence under a UV lamp indicates the presence of enterococci. The development of fluorescence after 28 hours should be disregarded.

5.8 Nutrient Agar + MUG Test *(for E. coli in drinking or ground water)*

To confirm the presence of *E. coli*, transfer the filter containing the coliform colony/colonies to the NA+MUG medium. See note below.

Nutrient Agar + MUG medium is available commercially. Prepared medium must be autoclaved in 100-mL volumes at 121°C for 15 minutes. MUG may be added to medium before autoclaving. The final MUG concentration must be 100 µg/ml and the final pH must be 6.8 ± 0.2.

If sterile medium is stored, the medium must be refrigerated in petri dishes, in a plastic bag or tightly closed container, and used within two weeks. Before use, refrigerated sterilized medium must be incubated overnight at room temperature; plates with growth must be discarded.

QC Run positive and negative controls for each new lot or batch of media. Filter or spot-inoculate controls onto a membrane filter on M-Endo LES agar or M-Endo broth or agar and incubate at 35°C for 24 hours. Then transfer the filter to Nutrient Agar + MUG and incubate at 35°C for another four hours. The results must be read and recorded.

Note: The membrane filter containing coliform colony/colonies must be transferred from the total coliform medium (m-Endo or LES Endo) to the surface of Nutrient Agar + MUG medium. Each sheen colony location should be properly marked on the lid (mark the lid and the base with a realignment line to pinpoint the location) so that the same colony or colonies can be verified for Total coliforms. A portion of the colony may be transferred with a needle to the total coliform verification test before transfer to Nutrient Agar + MUG or after the four-hour incubation time. Another way is to swab the entire membrane filter surface with a sterile swab after the 4-hour incubation on the NA+MUG medium (check for fluorescence first) and transfer to a total coliform verification test.

The Inoculated medium must be incubated at 35 ± 0.5°C for four hours and fluorescence is checked using an ultraviolet lamp (366 nm) with a 6-watt bulb in a darkened room. Any amount of fluorescence in a halo around a sheen colony must be considered positive for *E. coli*.

5.9 *Heterotrophic Plate Count* *(for enumerating heterotrophs in drinking and reagent-grade water)*

The Pour Plate Method (SM 9215B) or the SimPlate Method is approved for determining compliance with 40 CFR 141.74(a)(1). See also Appendix C. This method should also be used for testing reagent grade water.

The following table lists the different media for each HPC technique including the required final pH.

Table 5.9.1: HPC Media and final pH

Method	Medium	Final pH
Pour Plate	Plate count agar (aka Tryptone glucose yeast agar)	7.0 ± 0.2
	R2A agar	7.2 ± 0.2
Spread Plate	PCA	7.0 ± 0.2
	R2A agar	7.2 ± 0.2
Membrane Filter	R2A agar	7.2 ± 0.2
SimPlate	Multiple Enzyme Substrate	7.0 ± 0.3

Refrigerated medium may be stored in screw-capped bottles or screw-capped tubes for up to 6 months or in petri dishes for up to 2 weeks. Prepared R2A plates may be stored for up to one week

For most potable water samples, countable plates can be obtained by plating 1.0 ml and/or 0.1 ml of the undiluted sample. Dilutions may not be necessary for SimPlate, which has a counting range up to 738/ml. At least duplicate plates per dilution should be used.

QC Each batch or flask of agar must be checked for sterility by pouring a final control plate. Data must be rejected if control is contaminated.

QC In the case of laboratories having more than one analyst, each analyst must count the total colonies on a plate from a positive water sample at least once per month. The analysts' colony counts must agree within 10%.

5.9.1 Pour Plate Method

The melted agar must be tempered at 44-46°C in waterbath before pouring. Melted agar must be held no longer than three hours. Sterile agar medium must not be melted more than once. The sample must be aseptically pipetted onto the bottom of a 100 mm x 15 mm petri dish (100 x 15 mm or 90 x 15 mm), then 12-15 ml of the tempered melted (44-46°C) agar is poured into each petri dish. The sample and melted agar must be mixed carefully to avoid spillage. After agar plates have solidified on a level surface, the plates must be inverted and incubated at 35 ± 0.5°C for 48 ± 3 hours. Plates should be stacked no more than four high and arranged in the incubator to allow proper air circulation and to maintain uniform incubation temperature. Proper humidity should also be maintained to prevent spreader growth (for excessive humidity) and to avoid excessive drying (more than 15% by weight) during 48 hours of incubation. See note below for counting procedure.

5.9.2 Spread Plate Method

15 ml of agar medium must be poured into a petri dish (100x15 mm or 90x15 mm) and allowed to solidify. 0.1 or 0.5 ml of the sample or dilution must be pipetted onto the surface of the solidified agar then spread over the entire surface using a sterile bent glass rod. The inoculum must be absorbed completely by the agar before the plate is inverted and incubated at 20-28 °C (room temperature) for 5-7 days.

Note: For both Pour Plate and Spread Plate Techniques, colonies must be counted manually using a dark field colony counter. In determining sample count, laboratories must only count plates having 30 to 300 colonies, except for plates inoculated with 1.0 ml of undiluted samples, in which case, counts less than 30 CFUs are acceptable. (Fully automatic colony counters are not suitable for counting because the small size and number of colonies observed in potable water

samples maybe missed).

5.9.3 **Membrane Filter Technique**

The volume to be filtered must yield between 20-200 colonies. The filter is transferred to a petri dish containing 5 ml of solidified R2A medium and incubated at 20-28 °C (room temp) for 5-7 days. If plates with loose fitting lids are used, plates must be placed in a humidified container to prevent drying out. Colonies must be counted using a stereoscopic microscope at 10-15X.

5.9.4 **SimPlate Method** (IDEXX Laboratories, Inc.).

Unit Dose (for a single sample): 10-ml of test sample is added to a test tube containing dehydrated SimPlate medium. The dissolved medium should be poured evenly onto a plate containing 84 small wells (provided by the manufacturer). Excess fluid is absorbed by a pad in the plate. A dilution can also be made by using 9 ml sterile diluent (DI water, distilled or buffered water) and 1 ml of test sample. The plate should be inverted and incubated at 35 ± 0.5 °C for 45-72 hours. Bacterial density is determined by counting the number of wells that fluoresce under a 365 nm UV lamp and converting the obtained value to a Most Probable Number using the Unit Dose MPN Table. If 10 ml undiluted sample is used, the MPN is read off the chart. If 1-ml sample is used, multiply the MPN/ml value by the dilution factor of 10.
Multiple Dose (for 10 samples of 1 ml each): Reconstitute the SimPlate medium by adding 100-ml sterile diluent. Shake to dissolve. 1 ml test sample is pipetted onto the center of the plate containing 84 wells, followed by 9 ml of the reconstituted medium. Gently swirl the plate to mix and evenly distribute the mixture into the 84 wells. Invert and incubate as above and count using the Multi-Dose MPN Table supplied by the manufacturer. If the dilution of the sample is made, multiply the MPN/ml value from the chart by the dilution factor.

QC Re-verify pH for each new lot of SimPlate media: 7.0 ± 0.3

QC Perform sterility check for each new lot of media or diluent

QC Perform autofluorescence check for each new lot of media and SimPlate tray

QC Run positive and negative (media only) controls for each batch of samples and for each new lot of media

5.10 **Coliphage** [under proposal]

6. Sample Collection, Handling, and Preservation

Paragraphs 6.1 – 6.5 apply to laboratories that collect samples. However, laboratories should ensure that all samples are properly collected.

6.1 Sample Collector

The sample collector must be trained in aseptic sampling procedures and, if required, approved by the appropriate regulatory authority or its designated representative.

6.2 Sampling

Drinking water: Samples must be representative of the water distribution system. Water taps used for sampling should be free of aerators, strainers, hose attachments, mixing type faucets, and purification devices. Cold water taps should be used. The service line must be cleared before sampling by maintaining a steady water flow for at least two minutes (until the water attains steady temperature). At least 100 ml of sample must be collected, allowing at least a 1-inch air space to allow proper mixing of the sample. Immediately after collection, a sample information form should be completed (see paragraph 6.5 for the required information). For chlorinated water, adequate sodium thiosulfate ($Na_2S_2O_3$) must be added to the container to neutralize any residual chlorine.

Source Water: Source water samples must be representative of the supply source, collected not too far from the point of intake but at a reasonable distance from the bank or shore. The volume obtained should be sufficient to perform all the tests required.

Ground Water (for Coliphage analysis under Ground Water Rule (GWR)): At least 100-ml sample is required for the assay of either the somatic coliphages or the male specific (F+) coliphages. Larger volume (e.g. 200 ml) is recommended to allow for re-testing in case controls fail.

6.3 Sample Icing

Drinking water: Samplers are encouraged, but not required, to hold drinking water samples at refrigerated temperature (<10°C) during transit to the laboratory.

Source water: Source water samples under the Surface Water Treatment Rule (SWTR) must be transported at <10°C (see SM, 9060B). However, frozen samples should be rejected.

Ground water (for Coliphage analysis under GWR): Samples should be shipped at <10°C using iced water, Blue ice, ice gel, etc. to maintain temperature. Store at 1-5 °C and do not freeze.

QC For SWTR and Coliphage samples, the temperature upon receipt must be recorded. Samples with temperature >10°C should be flagged unless it is delivered to the laboratory within 2 hours of collection.

6.4 Sample Holding/Travel Time

Drinking water: All drinking water samples analyzed for Total coliforms, Fecal coliforms, or *E. coli must* not exceed 30 hours (40 CFR 141.21(f)(3)) from the time of collection to incubation. Samples may be refrigerated overnight but should be analyzed within 30 hours of collection. No sample exceeding this holding time should be analyzed. Recollection should be requested.

Drinking water samples for heterotrophic count should have a total collection/transit-to-analysis time of 8 hours or less. (40 CFR 141.74(a)(1)). Refer to SM 9215A for storage requirements and limitations.

Surface water: Collection to incubation for samples from surface water sources for microbiological analysis (Total and Fecal coliforms) must not exceed eight hours (40 CFR 141.74(a)(1)).

Ground water for *E. coli* and Enterococci (under GWR): The time between sample collection and incubation must not exceed 30 hours.

Ground water for coliphage analysis: Sample collection to incubation must not exceed 48 hours.

Sewage water for coliphage analysis: Sample collection to analysis of QC spiking suspensions may not exceed 24 hours unless re-titered and titer has not decreased by more than 50% in which case, the sample can be stored for up to 72 hours.

6.5 *Sample Information Form*

After collection, the sampler must complete the sample information form, write in indelible ink, the following information for compliance samples:

- Name of system (public water system site identification number, if available)
- Sample identification (if any)
- Sample site location
- Sample type (e.g., routine distribution sample, repeat sample [include date of original sample and repeat site, e.g. upstream, downstream, etc.], raw or processed water, other special purpose sample)
- Date and time of collection
- Analysis required
- Disinfectant residual
- Name of sampler and organization (if not the water system)
- Sampler's initials
- Person(s) transporting the samples from the system to the laboratory (if not the sampler)
- Transportation condition (e.g., <10°C, protection from sunlight). If a commercial shipper was used, shipping records must be available.
- Any remarks

6.6 *Chain-of-Custody*

Sample collectors and laboratories must follow applicable State regulations pertaining to chain-of-custody.

7. Quality Assurance

7.1 A written QA plan should be prepared and followed. It should be available for inspection by the certification officer. A laboratory that wishes to perform additional QA beyond what is in this manual may refer to Standard Methods, Section 9020, Quality Assurance/Quality Control (20th ed.). See Appendix B for the Criteria for a QA Plan.

Each laboratory shall have on file and available for inspection, a written description of the current laboratory quality control program. Such written description shall outline the procedures which the laboratory will use in meeting the quality control requirements. Each personnel involved with the laboratory operation (management, supervisors, analysts) should participate in developing the quality control program. Each analyst should review the quality control program, sign and maintain a schedule of review for the CO to inspect during the onsite.

The QA plan should specify that a laboratory that performs its own calibration of equipment and supplies should have a Standard Operating Procedure (SOP) available for review.

7.2 A record of analytical control tests and quality control checks on media, materials, and equipment shall be prepared by the laboratory and retained for at least five years.

7.3 There shall be available at all times, in the immediate work area, the current QA Plan, QC Policy manual, the laboratory SOP manual showing the most recent supervisory reviews, recent edition of the Standard Methods book and other pertinent manuals and books, for easy access and referral.

7.4 Only the laboratory manager or supervisor shall make changes in laboratory procedures and those changes shall comply with the current approved edition of *Standard Methods* and Federal regulations and be effective only when put in writing.

QC A yearly review of the QA plan (by laboratory manager and supervisor), Laboratory QC policies and SOP manual (by supervisor, analysts, technicians). must be done by all laboratory personnel involved with the analytical aspect of laboratory operation. The schedule for review should be properly documented with signatures and dates of review.

QC A laboratory should successfully analyze at least one set of Performance Evaluation (PE) or Proficiency Testing (PT) water microbiology samples once every 12 months for each method that the laboratory wants to be certified for and/or each method for which the laboratory has received certification or interim approval. The PE samples should be obtained from accredited providers. Read added explanation below (Paragraph 7.5 and Table 7.1).

7.5 For methods used to test the presence or absence of an organism in a sample, each set of Proficiency Testing should contain 10 samples in either a lyophilized, dehydrated or aqueous form. The set should include samples in various combinations containing Total coliforms, Fecal coliforms, E. coli and non-coliforms. There should be at least one blank. Each set should be used only with a single analytical method. To be acceptable, a laboratory should correctly analyze a minimum of nine of the ten samples (i.e. One false-positive result is allowed). However, ***just one false-negative will result in an unacceptable study.***

The following table identifies the methods that may be sufficiently similar to allow a laboratory to be certified for more than one method upon successful completion of a single set of PE samples.

Table 7:1 Specific Methods Requiring PT Analysis

Method Category	Specific Method *
MTF	LTB or P-A Broth → BGLB and EC / EC+MUG
MTF	A-1 Broth (Fecal coliform, SWTR only)
Enzyme Substrate	Colilert or Colilert 18
Enzyme Substrate	Colisure
Enzyme Substrate	Readycult or Fluorocult LMX
Enzyme Substrate	E*Colite
Enzyme Substrate	Colitag
MF	M-Endo or LES-Endo → BGLB and EC / EC+MUG or NA+MUG
MF	MI Medium
MF	Coliscan
MF	m-ColiBlue24
MF	Chromocult
MF	mFC Agar (Fecal coliform, SWTR only)
HPC	PCA
HPC	SimPlate

* A separate set of proficiency test samples is recommended for each cell. A single set of PT samples would cover every method within the same cell.

8. Records and Data Reporting

8.1 Legal Defensibility

Compliance monitoring data must be made legally defensible by keeping thorough and accurate records. The QA plan and/or SOPs must describe the policies and procedures used by the facility for record retention and storage. If samples are expected to become part of a legal action, chain-of-custody procedures should be used.

8.2 Maintenance of Records

Public water system are required to maintain records of microbiological analyses of compliance samples for five years (40 CFR 141.33). The laboratory should keep easily accessible records for at least five years or until the completion of the next certification data audit. The client water system should be notified before disposing of their records so that they may request copies if needed. This includes all raw data, calculations, and quality control data. These data files may either be hard copy, microfile or electronic. Electronic data should be backed up by protected tape or disk or hard copy. If the laboratory changes its computer system, provision should be made for transferring old data to the new system so that it remains retrievable within the time frames specified above. Data which is expected to become part of a legal action may need to be maintained for a longer period. Check with your legal counsel concerning this.

8.3 Sampling Records

Data should be recorded in ink. Any changes should be lined through but such that original entry is still visible. Changes should be initialed and dated The following information should be readily available in a summary or some other form of record:

- Sample information form (see 6.5 above)
- Date and time of sample receipt
- Name or initial of laboratory person receiving the sample;
- Rejection criteria for invalid samples should be noted such as:
  - Excessive holding/transit time (over 48 hours drinking water, 0ver 24 hours unrefrigerated source water)
  - Excessive temperature variance (boiling hot, frozen)
  - Unacceptable container (unsterile jars or bottles)
  - Insufficient volume (<100 ml)
  - Presence of excessive chlorine or other disinfectant (noticeable odor)
  - Contamination (e.g. motor oil, cleanser, powder, etc.)
- Drinking water samples > 30 hours but < 48 hours, surface water samples > 8 hours but < 24 hour old should be noted (or per laboratory policy regarding these type of samples. See section 6.4)
- Request for new or repeat sample should also be recorded.

8.4 *Analytical Records*

Data should be recorded in ink with any changes lined through such that original entry is still visible. Changes should be initialed and dated. The following information must be readily available in a summary or other form of record:

- Laboratory sample identification
- Date and time analysis begins
- Initial of analyst (If analysis was not performed at the receiving laboratory, the name and address of the testing laboratory and the analyst initial)
- Analytical technique or method used
- All items marked QC
- Results of analyses

8.5 Data Reporting:

The original or true duplicate of the results of the tests or analyses shall be signed by the laboratory manager and sent promptly to the person who requested such tests or analyses. Whenever a Certified, Provisional, or Decertified laboratory refers samples to another laboratory for analyses, the person requesting the analyses or tests shall receive the original laboratory report or a true duplicate of that report on the form of the laboratory that performed the tests or analyses. Report forms include the following information:

- IDEM Laboratory number
- Identification of sample number
- Date, time, and specific location of sampling
- Sample type (e.g., routine distribution sample, repeat sample [include date of original sample and repeat site, e.g. upstream, downstream, etc.], raw or processed water, other special purpose sample)
- Initials of sampler/organization submitting sample
- Free chlorine residual (if applicable)
- Date and time of sample receipt
- Date and time of sample analysis
- Identification of person performing the analysis
- Analytical method used
- Results of analysis
- Include fecal/*E. coli* result if total coliform positive
- Date analysis completed
- Analyst(s) initials

8.6 Preventive Maintenance

Laboratories must maintain preventive maintenance and repair activity records for all instruments and equipment (including pH and conductivity meters, analytical balances, incubators, refrigerators, autoclaves, water baths, and reagent grade water system). Records must be kept for five years and readily available for inspection.

9. Action Response to Laboratory Results

9.1 Total Coliform-Positive Cultures

- All Total coliform-positive cultures must be tested for presence of either fecal coliforms or *E. coli.*

- The Total coliform-positive cultures are from the confirmed phase of the MultipleTube Fermentation Technique and P-A Coliform Test or results from the verified test for the Membrane Filtration Technique (m-Endo or LES-Endo). Fecal coliform and/or *E. coli* positive results are considered Total coliform positive cultures.

9.2 Notification of Positive Results

- For the Total Coliform Rule, laboratories must promptly report (within 24 hours) all positive Total coliform, Fecal coliform, or *E. coli* compliance sample results to the IDEM Drinking Water Branch so that appropriate follow-up actions (like repeat sampling) can be conducted (CFR 141.21(b) & (e), 40 CFR 141.31, etc).

- If any sample is fecal coliform or E.coli positive, "the system must notify the State (IDEM) by the end of the day when the system is notified of the test result, unless the system is notified of the result after the State office (IDEM) is closed, in which case the system must notify the State before the end of the next business day." (40 CFR 141.21(e)(1)).

- All data should be reported to IDEM and local authorities within 40 days.

9.3 Notification of Non-Coliform Interference

- For the Total Coliform Rule, laboratories must promptly notify the proper authority (usually the water system) when results indicate that high levels of noncoliforms may have interfered with the Total coliform analysis (40 CFR 141.21(c)(2). IDEM Drinking Water Branch should be notified no later than the next business day

APPENDIX A
Definitions and Abbreviations

Accuracy: A measure of the closeness of an individual measurement or the average of a number of measurements to the true value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations.

Analyst: Any person who meets the state qualification requirements for education, training and performance of tests and methodologies used in analyzing water samples submitted to the laboratory

Analytes: In water microbiology laboratory, the microorganisms or contaminants being analyzed. E.g., Total coliforms, E.coli, etc.

Auditor: A person who evaluates laboratories to determine their status for certification. This person should be an experienced professional, has effective communication skills, experienced in quality assurance, and analytical techniques being evaluated, and familiarity with the drinking water regulations and this manual.

Bachelor's degree or Equivalent: A college degree with an equivalent 30 semester hours in a specific discipline. Equivalent is at least four years of experience in a specific scientific discipline.

Bias: The systematic or persistent distortion of a measurement process which causes errors in one direction.

Certification: A status of approval granted to an environmental laboratory, which meets the standards of the ISDH and USEPA.

Certification Officer (CO): A State or Federal laboratory auditor who has passed the NERL certification officers training course in chemistry and/or microbiology. This person is designated by ISDH to survey and evaluate environmental laboratories for compliance with Federal and State Standards and make recommendations on the certification status of a laboratory.

Certified Thermometer: A thermometer that has documentation showing that it has been compared against an appropriate National Institute of Science and Technology (NIST) thermometer for its temperature range.

CFR ***(Code of Federal Regulations)***: A compilation of rules and regulations revised each time a regulation is promulgated by several federal government agencies including the Administrator of the United States Environmental Protection Agency. It is published every year in July.

CFU: Colony forming units, a more precise term for 'colonies'

Chromogenic/Fluorogenic Substrate Test: A method for determining the presence of Total coliforms and *E. coli*. using a chromogenic substrate which when cleaved by Total coliforms produce a color change and a fluorogenic substrate which when cleaved by E. coli produce fluorescence under an UV light. (See MMO-MUG).

Commissioner: The Commissioner of the Indiana State Department of Health.

Community Water System: Water supply system with 15 service connections used by at least 25 year-round residents.

Comparability: The agreement of data generated by different systems. Comparability of data is assured by following standard analytical procedures and calculating and reporting all data in generally accepted units.

Completeness: A measure of the amount of valid data obtained from a measurement system compared to the amount that was expected to be obtained under correct normal conditions. Completeness of data is dependent upon both field and laboratory personnel. Improper sample collection, sample contamination, and out-of-control analytical procedures can cause the loss of data.

Compliance samples: Public water system samples monitored for contaminants under the SDWA.

Confirmation Test: Test for the presence of a contaminant through the use of another test or method based on a different scientific principle from the original method. This is usually done for Membrane Filtration presumptive growths.

Confluent Growth: Bacterial growth that covers the entire filtration area of the filter with no discrete colonies.

Data audit: A qualitative and quantitative evaluation of the documentation and procedures associated with measurements to verify acceptability of the results.

Data Reduction: The process of transforming the number of data items by arithmetic or statistical calculations, standard curves, concentration factors, etc. and collation into a more useful form. DR is irreversible and generally results in the loss of detail.

Drinking Water Laboratory: A laboratory that analyzes samples as part of compliance monitoring for a public water supply.

Environmental Water Laboratory: A facility that analyzes the microbiological quality of environmental water samples such as public water supplies, surface water, ground water, recreational water, wastewater, air, or land using EPA approved methods

EPA: Also, USEPA. The United States Environmental Protection Agency

Holding time: The period covering the time of collection of a sample until initiation of analysis.

IAC: Indiana Administrative Code

IDEM: Indiana Department of Environmental Management.

Invalidation: Action response to Membrane Filtration Total coliform-negative or TNTC test results.

IPDWR: Indiana Primary Drinking Water Regulations

ISDH: Indiana State Department of Health.

Laboratory Pure Water: Distilled or deionized water which is free of contaminants that may interfere with the analytical test.

Laboratory Seeking Certification: An uncertified laboratory that has submitted an acceptable application and/or a laboratory holding a valid interim approval.

Matrix: Type of sample submitted for analysis. Example, drinking water, waste water, surface water, pool water, etc.

Maximum Contaminant Level (MCL): The maximum permissible level of a contaminant allowed in drinking water under the NPDWR delivered to any user of a public water system.

Membrane Filtration Method (MF): A method for determining presence or the bacterial count in a water sample in which a 100 ml volume of water is filtered through a membrane filter of optimum pore size for full bacterial retention. The filter is incubated in a prescribed media at appropriate time and temperature and examined for valid bacterial colonies which are counted and recorded as # per 100 ml of water sample. A "presence/absence" reporting format is used for compliance purposes.

MMO-MUG Test: Minimal Medium ortho-nitrophenyl-galactopyranoside (MMO)-methlumbelliferyl glucuronide (MUG) is a chromogenic-fluorogenic substrate incorporated into the media (in Colilert tests) to detect simultaneous specific enzymatic activities of Total coliforms and *E. coli*. Chromogens are compounds that turn a distinct color when cleaved by bacterial enzymes and fluorogens fluoresce when cleaved. A "presence/absence" reporting format is used for compliance purposes. MMO has become synonymous with any test using the chromogenic indicator system even though Colilert is the only test that uses the ONPG indicator for the presence of Total coliforms.

Most Probable Number (MPN): A method of reporting results of the coliform test by the multiple-tube fermentation procedure. This is an index of the number of coliform bacteria, more probably than any other number, would give the results shown by the laboratory examination; it is not an actual enumeration.

Multiple Tube Fermentation (MTF) a method for determining the presence of total coliforms in which a standard culture medium is inoculated with the sample volume, the tubes are incubated, and observed for gas production. A "presence/absence" reporting format is used for compliance purposes. MPN report format that gives an estimate of coliform density in the sample provides the best assessment of water treatment effectiveness and the sanitary quality of source water.

"Must": Denotes a mandatory requirement

National Primary Drinking Water Regulations (NPDWR)" found in 40CFR141, as amended, which were duly promulgated as regulations by the Administrator of the United States Environmental Protection Agency.

NELAC: National Environmental Laboratory Accreditation Conference

Non-community Water System a public water system, which has at least 15 service connections, used by nonresidents, or which regularly serves 25 or more nonresident individuals daily for at least 6 months per year. Non-transient, non-community system is used by the same 25 people (e.g., schools, churches, and factories). Transient, non-community system is used by different individuals (e.g., truck stops, campgrounds, hotels, restaurants, etc.)

NIST: National Institute for Standards and Technology

NVLAP: National Voluntary Laboratory Accreditation Program

Performance Evaluation (PE) sample / study: A sample or set of proficiency test samples provided to laboratories by accredited providers, in order to assess the performance of the laboratory to successfully analyze the samples within specified acceptance limits. The composition of the study material is unknown to the laboratory at the time of the analysis.

Precision: The repeatability of the measurement or degree of agreement between two measurements. Precision is best expressed in terms of standard deviation. Various measures of precision exist depending upon the prescribed similar conditions.

Presence/Absence (P/A)" a reporting format for all compliance monitoring. Bacteriological tests are interpreted and reported as "present" or "absent" after the appropriate confirmatory procedures are carried out.

Presence/Absence (P/A) Test: A total coliform test using the Clark's P/A broth formula. One 100 ml sample portion in a single culture bottle of P/A broth is used to obtain qualitative information concerning the presence or absence of coliforms. Results are interpreted as "present" or "absent" after appropriate confirmatory procedures are carried out.

Presumptive Culture or Tube: The positive result of an initial test to determine the presence of a contaminant in a given sample. It must be subjected to confirmation testing.

Provisional Certificate" a certification status granted to an environmental laboratory in order to allow time for the correction of deviations. While on provisional certification, an environmental laboratory may conduct compliance monitoring analysis for analytes for which it has been granted certification but must immediately notify its clients of its downgraded status and provide that information, in writing, on any report.

Public Water System: A water supply system for the provision of piped water for public consumption. Such system should have at least 15 service connections or regularly serves an average of at least 25 individuals daily at least 60 days out of the year.

QA Plan: A comprehensive plan detailing the aspects of quality assurance needed to adequately fulfill the data needs of a program. This document is required before the laboratory is certified.

Quality Assurance (QA): An integrated system of *management* activities involving planning, implementation, documentation, assessment, reporting and quality improvement to ensure that a process, item or service is of the type and quality needed and expected by the client.

Quality Control (QC): The overall system of *technical* activities that measures the attributes and performance of a process, items or service against defined standards to verify that they meet the established requirements. It includes operational techniques and activities used to substantiate the quality and validity of analytical data.

Reciprocity: The mutual recognition of a laboratory certification status between State certification authorities.

Representativeness: Expresses the degree to which data accurately and precisely represent a characteristic of a population, parameter variations at a sampling point, a process condition, or an environmental condition. Appropriate selection of sample site and sampling procedure is critical to obtaining a sample that is representative of the environment in which it is collected.

SDWA: The federally enacted Safe Drinking Water Act.

Sensitivity:

"Should": Denotes a guideline or recommendation.

Specificity:

State: In this manual, refers to the state of Indiana.

Standard Methods (SM): Standard *Methods for the Examination of Water and Wastewater*, American Public Health Association, approved edition as cited in the latest *Code of Federal Regulations*.

SOP (Standard Operating Procedure): A written document that details the method of an analysis or action to be followed. The prescribed techniques and procedures are officially approved as the method for performing certain routine or repetitive tasks.

SWTR: Surface Water Treatment Rule (See Appendix D)

TCR: Total Coliform Rule (See Appendix D)

TNTC (Too numerous to count): The result designated to a membrane filter culture that has grown so that the total number of bacterial colonies are too numerous (usually over 200) or not sufficiently distinct to obtain an accurate count (confluent growth). For source water samples, the test must be invalidated and repeat sample requested immediately. For drinking water samples, the culture may be subjected to verification tests first before invalidation.

USEPA United States Environmental Protection Agency.

Verification Test: A definitive test to ascertain the presence of a confirmed contaminant in a given sample.

APPENDIX B
I. General Outline of a Quality Assurance Plan

The Quality Assurance plan is a written description of the laboratory's management activities involving planning, implementation, documentation, assessment, reporting and quality improvement to ensure that a process, item or service is of the type and quality needed and expected by the client. It includes adhering to the QC procedures specified in the methods to ensure that routinely generated analytical data are valid and defensible, and of known and acceptable precision and accuracy. All laboratory personnel must be familiar with its contents. It should be updated as necessary by the QA manager. It should be brief, concise and easy to follow.

Note: Items listed in the QA Plan may be referenced to appropriate sections of this manual, the laboratory's SOP or other literatures like the Standard Methods book, Federal Registers, etc.

I. Introduction

- Introduces the general principle and policies under which the laboratory operates
- Includes the table of contents and the update policy of the plan.
- Includes a continuously updated list of amendments with dates of last revision.
- Includes a schedule of annual review or approval by key management with signatures and dates

II. Laboratory Organization and Responsibilities

- Outlines the chain of command and lines of responsibility of all laboratory personnel including the QA Manager/Director.
- Lists the key personnel responsible for internal audits and reviews of the implementation of the plan and its requirements.
- References the job descriptions and describes training including documentation of personnel proficiency for the methods they perform.

III. Quality Assurance Objectives

- Describes the laboratory's commitment to quality assurance and the process used to identify client's Data Quality Objectives (qualitative and quantitative specifications used to design a study that will limit uncertainty to an acceptable level).
- References the most current approved federal and state regulations and any other sources of information to be used.
- Expresses measurement of data in terms of precision, accuracy, representativeness, comparability and completeness.

IV. Sample Collection and Custody

- Describes the process used to identify sample, sampling procedures and location, required preservation and containers
- Defines sampling instructions made available to the collector
- Specify appropriate forms and logs to be completely filled out legibly with indelible ink or if available, hard copies of electronic data.
- Specifies the procedure to maintain the integrity of all samples from receipt through analysis to disposal (receiving, logging, storage, tracking).
- Specify criteria for shipping, holding time and preservation (e.g., pH, chlorine residual) requirements.
- Defines criteria for rejection and notification of sample originator, proper sample disposal and record keeping.
- Chain-of-Custody procedure applied for samples likely to be used for an enforcement action.

V. Calibration Procedures (may reference SOP)

- Includes procedures for the laboratory's calibration and maintenance of its own instruments, equipment and supplies.
- Specify type of calibration used for each method and frequency of calibration
- Describes standard source, age, storage, labeling

VI. Analytical Methods (may reference SOP)

- Lists and details Standard Operating Procedures performed in the laboratory.
- Ensure current copies of SOP manual are kept in the laboratory and in the QA Manager's files.
- Ensure that SOP's are reviewed annually and revised as changes are made with signatures and dates
- Cite the complete method manual and describe QC procedures required by the methods that must be followed.
- Details verification, confirmation and completed procedures and QC criteria (method validation)

VII. Data Handling (may reference SOP)

- Describes data reduction process (e.g. method of converting raw data to coliforms/100 ml)
- Describes data validation process (e.g. ensure accuracy of data transcription and calculations)
- Describes reporting procedures and format (include a copy of the report form and fill-out instructions)
- Describes data verification process (e.g., person responsible for reviewing final report)
- Describes procedure for data corrections
- Describes data comparability checks

VIII. Quality Control Checks and Frequency of Use (may reference SOP)

Describes and state frequency for the following:

- Positive and negative culture controls
- Confirmation/verification of presumptive total coliform-positive samples
- Sterility controls
- Performance evaluation (Proficiency Test) samples
- Software validation

IX. Performance Evaluation and System Audits (may reference SOP)

- Lists schedules of internal system and data quality audits
- Lists schedules of external system and data quality audits and inter-laboratory comparisons
- Describes corrective actions for audits

X. Preventive Maintenance Procedures and Schedules

- Describes location of instrument manuals and schedules
- Documentation of routine equipment maintenance
- Describes availability of spare parts
- Describes list of maintenance contracts or service

XI. Corrective Action Contingencies

- Describes troubleshooting action plans to unacceptable results from internal QC checks and PE results
- Personnel responsible for the various corrective actions
- Describes how corrective actions taken are documented and followed up

XII. Record Keeping Procedures

- Describes record keeping procedures and documentation of those procedures
- Lists length of storage, media type (electronic or hard copy)
- Describes security policy of electronic databases

XIII. Facilities and Environment

- A floor plan with detailed information on specifications (dimensions, usable space, counter space, special equipment areas, electrical and plumbing, air quality, ventilation, lighting, etc.), content (non-movable and moveable equipment), location of safety equipment, utilization of the space and duties performed in each area.
- Includes general and specific housekeeping practices, monitoring procedures and records.

XIV. QA Reports to Management

- Includes the management's periodic review of the adequacy of the QA program for suitability and effectiveness.
- Management assessment reports summarize the laboratory's operational status and may include some or all of the following items:
  - Corrective actions
  - QA deviations or deficiencies
  - Client complaints
  - Internal audit summaries
  - External audit summaries
  - Proficiency test results
  - Certification status

XV. Safety Plan (may be written as a separate manual)

- All aspects of safety within the facility should be accounted for within this plan.
- Details all safety issues including facility design, safety equipment and location, personnel safety training, personnel responsibility (management, safety officer and personnel) and action plans
- Describes a system of reporting safety-related issues (e.g., safety officer, management, fire and /or police, etc.)
- Description of routine safety practices (protective clothing or equipment), general laboratory safety rules, identification of all materials, safe handling of all media, chemicals or other supplies and general housekeeping practices.
- Evacuation plans in case of fire or other disasters as well as list of contacts for various safety problems.

APPENDIX B
II. RECOMMENDED SOP FORMAT

The following comprehensive SOP format is recommended by the Microbiology Certification Program. Each analytical method SOP used in the Water Microbiology Laboratory should contain these sections and designated in the following order (if the section is not applicable or not necessary, it should be stated as such):

I. Principle/ Scope/ Application

II. Summary of Method, Detection Limit

III. Matrix/ Sample Requirements/ Sample Collection, Preservation, Storage

IV. Equipment and Supplies/ Materials

V. Reagents/ Media

VI. Quality Control/ Standards

VII. Procedure (include waste management)

VIII. Data Analysis / Calculations/ Interpretation/ Reporting

IX. Method Performance/ Procedural Notes/ Limitations

X. Author and Date

XI. References

Outline of SOP pertaining to the laboratory's calibration of its own equipment and supplies:

I. Principle/Scope and Application

II. Instrument Make/Model/Serial Number

III. Reagents and Standards

IV. Procedure

V. Data Analysis and Calculations

VI. Maintenance/ Preventive Maintenance (this can be referenced to the QA Plan)

VII. Reference

Unless referenced to the QA/QC Manual, all significant information about the method should already be described in the SOP without having to read another manual or book. For example, "Principle - See SM 9223A Section 1" is not acceptable.

APPENDIX C

Table 1

List of references for methods used in the examination of drinking water
(Most of these methods are contained in the 18th, 19th & 20th edition of *Standard Methods for the Examination of Water and Wastewater*).

Organism	Method	Citation
Total Coliform	Fermentation Techniques	9221A,B
	Membrane Filtration Techniques[1]	9222A,B,C
	Presence-Absence Test	9221D
	ONPG-MUG Test[2]	9223
	Colisure™ Test [3]	
	E*Colite® Test[4]	
	m-ColiBlue24® Test[5]	
	Readycult Test	
	Colitag Test	
Fecal Coliform	EC Broth	9221E
	A-1 Method	9221E
	Mfc	9222D
E.coli	EC+MUG Broth**	9221E
	NA+MUG Agar**	9221E
	ONPG-MUG Test	9223
	Colisure™ Test	
	E*Colite® Test	
	m-ColiBlue24® Test	
	Readycult Test	
	Chromocult	
	Coliscan	
	MI Medium	
Heterotrophs	Heterotrophic Plate Count	9215B

**With appropriate concentrations of MUG added.

[1]MI agar may also be used. Preparation and use of MI agar is set forth in the article "New medium for the simultaneous detection of total coliform and *E. coli* in water" by Brenner, K.P., et. al., 1993, Appl. Environ. Microbiol. 59:3534-3544. Also available from the Office of Water Resource Center (RC-4100), 401 M. Street SW, Washington, D.C. 20460, EPA/600/J-99/225.

[2]The ONPG-MUG Test is also known as the MMO-MUG or Colilert Tests.

[3]A description of the Colisure™ Test may be obtained from the IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092. The Colisure™ Test may be read after an incubation time of 24 hours.

[4]A description of the E*Colite® Test, "Presence/Absence for Coliform and *E. coli* in Water," is available from Charm Sciences, Inc., 36 Franklin Street, Malden, MA 02148-4120.

[5]A description of the m-ColiBlue24® Test is available from the Hach Company, 100 Dayton Avenue, Ames, IA 50010.

APPENDIX C

Table 2

EPA approved methods, applicable regulations and SM citations
Revised July 2003

Approved Methods	Analyte	Media	TCR (Detect)	SWTR (Count)	GWR (Detect)	Std Method cited
Fermentation Broth Method	TC	LTB → BGLBB	X	X		SM 9221B,C
	TC	P-A Broth → BGLBB	X			SM 9221D
	FC	LTB or P/A Broth → EC Broth	X	X		SM 9221B,D SM 9221E
	FC	A-1 Broth		X		SM 9221E
Enzyme Substrate Method	TC / *E. coli*	Colilert®, Colilert 18®	X	X	X	SM 9223
	TC / *E. coli*	Colisure®	X		X	SM 9223
	TC / *E. coli*	Readycult® or Fluorocult LMX®	X			
	TC / *E. coli*	E*Colite® Test	X		X	
	TC / *E. coli*	Colitag®	X			
	E. coli	LTB, P/A Broth, M-Endo → EC-MUG	X		X	SM 9221B,D SM 9222B SM 9221F
	TC / *E. coli*	Quanti-Tray / Quanti-Tray 2000 (MPN)	X	X		
Membrane Filter Method	TC	M-Endo or LES-Endo → LTB, BGLBB	X	X		SM 9222B,C
	TC / *E. coli*	MI Medium	X	X	X	
	TC / *E. coli*	m-ColiBlue 24®	X		X	
	TC / *E. coli*	Chromocult®	X			
	TC / *E. coli*	Coliscan®	X	X		
	FC	M-Endo Medium → EC Broth	X	X		SM 922B SM 9221E
	FC	mFC		X		SM 9222D
	E. coli	M-Endo or LES-Endo → NA -MUG	X		X	SM 9222B SM 9222G
Heterotrophic Plate Count (for Heterotrophic Bacteria)*						
Pour Plate Method	Plate Count Agar			X		SM 9215B
Spread Plate Method	R2A			X		SM 9215C
Multiple Enzyme Method	SimPlate®			X		
				X		

TC Total coliform
FC Fecal coliform
E. coli *Escherichia coli*
TCR Total Coliform Rule
SWTR Surface Water Treatment Rule – Goal: <100 CFU / 100 ml Total coliform
< 20 CFU / 100 ml fecal coliform
*HPC HPC maybe measured in lieu of disinfectant residual (< 5% of samples may have non-detectable disinfectant residual) for distribution systems – Goal <500 CFU/ml

APPENDIX D

TOTAL COLIFORM RULE (40 CFR 141.21 f)

Analytical methodology:

1. Standard volume is 100 ml regardless of method.
2. Public water system need only determine presence/absence of Total coliform.
3. One of the following analytical methods must be used for Total coliforms:
   a. Fermentation Technique
   b. Membrane Filter Technique
   c. P-A Test
   d. MMO-MUG Test - Colilert, Colisure, E*Colite, m-ColiBlue24, Readycult Coliforms 100 / Fluorocult LMX, Colitag or Membrane Filter Technique using Chromocult Coliform Agar

NOTES:

1) Sample collection to initiation of analysis may not exceed 30 hours.
2) Transport temps below 10 °C encouraged but not required.
3) Comparison rate must not exceed 10% between false+ and false- on 25 parallel studies of broths (Fermentation technique)
4) To detect gas production, media should cover inverted tubes at least 1/2 to 2/3 after sample is added. (Fermentation technique)
5) 10% of TC pos confirmed tubes need not be carried to completion. (Fermentation technique)
6) On P/A Coliform Test, 6x-formulation strength may be used if medium is filter-sterilized rather than autoclaved.
7) EPA strongly recommends that laboratories evaluate the false-positive and –negative rates for the method(s) they use for monitoring total coliforms (establish rates and matrix, drinking water or source water). If method has unacceptable rates, another method should be chosen.

4. [Reserved]
5. PWS must determine presence of Fecal coliform from lactose-positive presumptive culture:
   a. For MTF technique or P/A Coliform test
      1) Shake tube vigorously
      2) Transfer growth using sterile loop or stick into brilliant green lastose bile (BGLB) broth and EC medium to determine presence of total and fecal coliforms, respectively.
   b. For Membrane Filtration technique, use one of the following methods:
      1) Using sterile forceps, transfer positive culture membrane by curling membrane into EC medium, or
      2) Swab entire membrane filter surface with sterile cotton swab and inoculate EC medium (do not leave swab in the medium), or
      3) Inoculate individual colonies into the EC medium
   c. Incubate EC media at 44.5 +/- 0.2-°C water bath for 22-26 hrs
   d. Gas production in any amount in the inner fermentation tube indicates a positive fecal coliform test.
   e. PWS need only determine presence or absence of fecal coliforms (Fecal coliform density is not required).
6. PWS must determine presence of Escherichia coli in Total coliform-positive cultures in accordance with one of the following methods:
   a) Inoculate EC + 50 µg/mL MUG medium, incubate at 44.5 +/- 0.2 for 22-26 hrs then observe for fluorescence with a UV light (366 nm). Positive fluorescence indicates presence of *E. coli*.
   b) Inoculate nutrient agar + 100 µg/mL MUG, incubate at 35 °C for 4 (?) hrs and observe for fluorescence.
   c) Use Minimal Medium ONPG-MUG (MMO-MUG), incubate at 35 +/- 0.5 °C for 24 hrs then observe positive cultures for fluorescence. Incubate an additional 4 hrs for equivocal results and again test for fluorescence. Use hepes buffer instead of phosphate buffer.
   d) Colisure Test
   e) Membrane Filter method with MI agar
   f) E*Colite Test
   g) m-ColiBlue24 Test
   h) Readycult Coliforms 100 Presence/Absence Test
   i) MF Technique using Chromocult Coliform Agar, Colitag Test

7. A Total coliform positive, MUG negative MMO-MUG test may be further analyzed for the presence of E. coli by inoculating 0.1 ml of a 28-hr culture to EC+MUG medium.

8. List of references:

a. Safe Drinking Water Hotline 1-800-426-4791
b. EPA Drinking Water Docket, 1301 Constitution Avenue, NW., EPA West, Room b102, Washington, DC 20460 Telephone 202-566-2426
c. Office of Federal Register, 800 North Capitol Street, NW, Suite 700, Washington, DC 20408
d. Standard Methods for the Examination of Water and Wastewater, 18th edition (1992), 19th edition (1995), or 20th edition (1998). American Public Health Association, 1015 Fifteenth Street NW, Washington, DC 2005
e. MF Technique using MI Agar: Office of Water Resource Center (RC-4100T), 1200 Pennsylvania Avenue, NW, Washington, DC 20460, EPA/600/J-99/225 (Verification of colonies is not required).
f. Colisure Test: IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092 (Test maybe read after a 24 hour incubation period).
g. E*Colite Test: Charm Sciences, Inc., 36 Franklin Street, Malden, MA 02148-4120.
h. m-ColiBlue24 Test: Hach Company, 100 Dayton Avenue, Ames, IA 50010
i. Readycult Coliforms 100 P/A Test: EMD Chemicals, Inc. 480 South Democrat Road, Gibbstown, NJ 08027-1297, (800) 222-0342, adellenbusch@emdchemicals.com, www.emdchemicals.com.
j. MF Technique using Chromocult Coliform Agar: EMD Chemicals, Inc. (see info (i) above)

SURFACE WATER TREATMENT RULE (40 CFR 141.74(a))

Applies to PWS using SW or GW under the influence of surface water.

Criteria were established for avoiding filtration requirement. Two of these criteria involve quantitative microbiological parameters.

QUANTITATIVE MICROBIOLOGICAL CRITERIA

A. Source Water Samples
- Must be analyzed for Total or Fecal coliforms (no. of samples dependent upon population)
- Minimum of 1 sample per week Maximum of 5 samples per week
- In addition, samples are required on any days when turbidity levels exceed 1.0 ntu
- Total coliform level must not exceed: 100 CFU/100 ml
- Fecal coliform level must not exceed: 20 CFU/100 ml for more than 10% of samples taken over previous 6 month period, based upon monthly calculations

B. Distribution System
- No more than 5% of samples may have a non-detectable disinfectant residual
- Sample frequency same as for total coliforms, based upon calculation for 2 consecutive months
- HPC may be measured in lieu of disinfectant residual
- HPC levels must be less than: 500 CFU/ml to be equivalent to a sample containing a disinfectant residual

APPROVED MICROBIOLOGICAL METHODS

A. Source water
1. Total coliforms
   a. MF: m-Endo medium
      ml agar
   b. MTF: LTB/BGB media
      Colilert/Quanti-Tray

2. Fecal coliforms
   a. MF: mFC medium
   b. MTF: LTB/EC media
      A-1 medium (broth) - direct method
3. E. coli (not yet approved)

B. Distribution System
1. HPC: Aerobic pour plate - Plate count agar (Pour Plate agar)
   Simplate

Analytical requirements:
a. PWS must conduct analysis of pH and temperature in accordance with one of the methods listed
b. PWS must conduct analysis of total coliforms, fecal coliforms, heterotrophic bacteria and turbidity in accordance with one of the prescribed methods

Notes:
1) Sample collection to initiation of analysis may not exceed 8 hours. Transport temp must be below 10 °C.
2) At least 25 parallel samples must be analyzed between media (FermentationTechnique) when using different broths. Comparison rate of false-pos and false-neg must be less than 10%.
3) Fermentation test need not be carried to completion on 10% of all total coliform + confirmed tubes.

MF Method: Fecal coliform

1. Filter sample onto membrane
2. Place membrane on mFC medium
3. Incubate for 24 +/- 2 hr at 44.5 +/- 0.2 °C
4. Count blue colonies (any shade of blue)
5. Verification - same as fecal coliform test in broth media

*Specificity of test is related directly to the incubation temperature - use water bath or heat sink type incubator

MTF Method: Fecal coliform - A-1 medium

1. Inoculate tubed medium using MPN format
2. Incubate tubes for 3 hrs 35 +/- 0.5 °C
3. Transfer tubes to 44.5 +/- 0.2 °C water bath for an additional 21 hrs
4. Check for gas production
5. Determine MPN index and report MPN value per 100 ml

(Goal: <20 / 100 ml)

HPC: Aerobic Pour Plate Method (PCA)
1. Aseptically pipet sample in sterile petri dish (57 cm)
   - Run duplicate plates per dilution
2. Pour the tempered PCA medium into the dish and carefully mix with sample (tempered agar - crucial)
3. Allow agar to solidify and incubate inverted plates for 48 hrs at 35 +/- 0.5 °C
4. Count CFU and report as CFU/ml

* It is essential that the sterile melted medium be tempered in a water bath at 44-46 °C

Ground Water Rule (to be added after rule promulgation)

APPENDIX E

IMPORTANT TELEPHONE NUMBERS AND WEB SITES
(UPDATED 2011)

Indiana State Department of Health 2 North Meridian Indianapolis IN 46204	(317) 233-1325
ISDH Laboratory Services / Certification Office 550 W. 16th Street Indianapolis, IN 46202 Environmental Microbiology Laboratory Supervisor Certification Office (Microbiology)	 (317) 921-5500 (317) 921-5522 (317) 921-5523
ISDH Weights & Measures 525 North Shadeland Ave D3 Indianapolis IN 46219	(317) 356-7078
Indiana Department of Environmental Management Environmental Helpline	(800) 451-6027
IDEM Office of Water Quality Drinking Water Branch 100 N. Senate Ave. Indianapolis IN 46204	(317) 234-7430
EPA Safe Drinking Water Hotline	(800) 426-4791
EPA New Laboratory ID Numbers	(513) 569-7671

Links to Certified DW Labs (for each state)	www.epa.gov/safewater/labs/index.html
CFR	www.gpoaccess.gov/cfr/index.html
DW Regulations	www.epa.gov/ogwdw/regs.html
Methods	www.epa.gov/ogwdw/methods/methods.html
List of Indiana Certified Laboratories	http://www.in.gov/isdh/24859.htm or www.epa.gov/safewater/labs/index.html

REFERENCES

1. U.S. EPA Manual for the Certification of Laboratories Analyzing Drinking Water, Fifth Edition, EPA 815-R-05-004; January 2005 Chapter V Microbiology

2. Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998, American Public Health Association, 1015 Fourteenth Street NW Washington, DC 20005

3. Indiana Certification Standards for Drinking Water Microbiology Laboratories, 1998, ISDH Laboratory Resource Center, 635 North Barnhill Drive, Indianapolis, IN 46202

4. Federal Register 40 CFR 136, 141 and 143 October 23, 2002 (National Primary and Secondary Drinking Water Regulations, Methods Updates, Final Rule)

5. Federal Register 40 CFR 141 and 143 Wednesday December 1, 1999 (Final Rule on Revisions to Laboratory Certification Requirements) Internet http://www.epa.gov/fedrjstr

6. Information about drinking water regulations and laboratory certification, Labcert Bulletin: http://www.epa.gov/OGWDW/standards.html